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Analysis of Phosphonic Acids: Validation of Semi-Volatile Analysis by HPLC-MS/MS by EPA Method MS999

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Disclaimer

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Analysis of Phosphonic Acids: Validation of Semi-Volatile Analysis by HPLC-MS/MS by EPA Method MS999

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TECHNICAL REPORT

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Overview and Objectives

The Environmental Protection Agency's (EPA) Region 5 Chicago Regional Laboratory (CRL) developed a method titled *Analysis of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid, and Pinacolyl Methylphosphonic Acid in Water by Multiple Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry: EPA Version MS999*. This draft standard operating procedure (SOP) was distributed to multiple EPA laboratories and to Lawrence Livermore National Laboratory, which was tasked to serve as a reference laboratory for EPA's Environmental Reference Laboratory Network (ERLN) and to develop and validate analytical procedures.

The primary objective of this study was to validate and verify the analytical procedures described in *EPA Method MS999* for analysis of the listed phosphonic acids and surrogates in aqueous samples. The gathered data from this validation study will be used to: 1) demonstrate analytical method performance; 2) generate quality control acceptance criteria; and 3) revise the SOP to provide a validated method that would be available for use during a homeland security event. The data contained in this report will be compiled, by EPA CRL, with data generated by other EPA Regional laboratories so that performance metrics of *EPA Method MS999* can be determined.

LLNL Verification of Procedures

Task 1: Verification of Instrument Conditions

For this study, a Waters Micromass Quattro *micro* API triple quadrupole mass spectrometer (Serial Number QAA594) coupled to a Waters 2795 liquid chromatograph was

utilized for analysis. To verify instrument conditions, individual standards of diisopropyl methylphosphonate (DIMP; CAS # 1445-75-6), ethyl hydrogen dimethylamidophosphate (EHDMAP; CAS # 2632-86-2), ethyl methyl phosphonic acid (EMPA; CAS # 1832-53-7), isopropyl methylphosphonic acid (IMPA; CAS # 1832-54-8), and pinacolyl methylphosphonic acid (PMPA; CAS # 616-52-4), along with the surrogate compounds methylphosphonic acid-D_3 (MPA-D_3), pinacolyl methylphosphonic acid-$^{13}C_6$ (PMPA-$^{13}C_6$), and diisopropyl methylphosphonate-D_{14} (DIMP-D_{14}) were prepared at concentrations of approximately 100 µg/mL in water/acetonitrile (50/50, v/v) + 0.1% formic acid. These individual standards were infused at 20 µL/min using an external Harvard Syringe Pump Model 22 (Harvard Apparatus, Holliston, MA) and ionized in both positive and negative ion mode electrospray ionization (ESI). The initial tune file used for validating the ionization of each phosphonic acid was as described in the *EPA Method MS999* SOP (**Table 1**). All instrument conditions, including voltages (capillary, cone, extractor, and RF lens), temperature (source, desolvation), gas flows (desolvation, cone), energies (ion, entrance, collision, and exit), resolutions (for low and high mass), multiplier, reaction mode and optimal ions for analysis were optimized and recorded. The optimized parameters are shown in **Table 1**. All ions previously identified in the *EPA Method MS999* SOP were confirmed and the following transitions from parent to product ion are listed in **Figure 1** and **Table 2**.

Figure 1: Analyte and Surrogate Structures

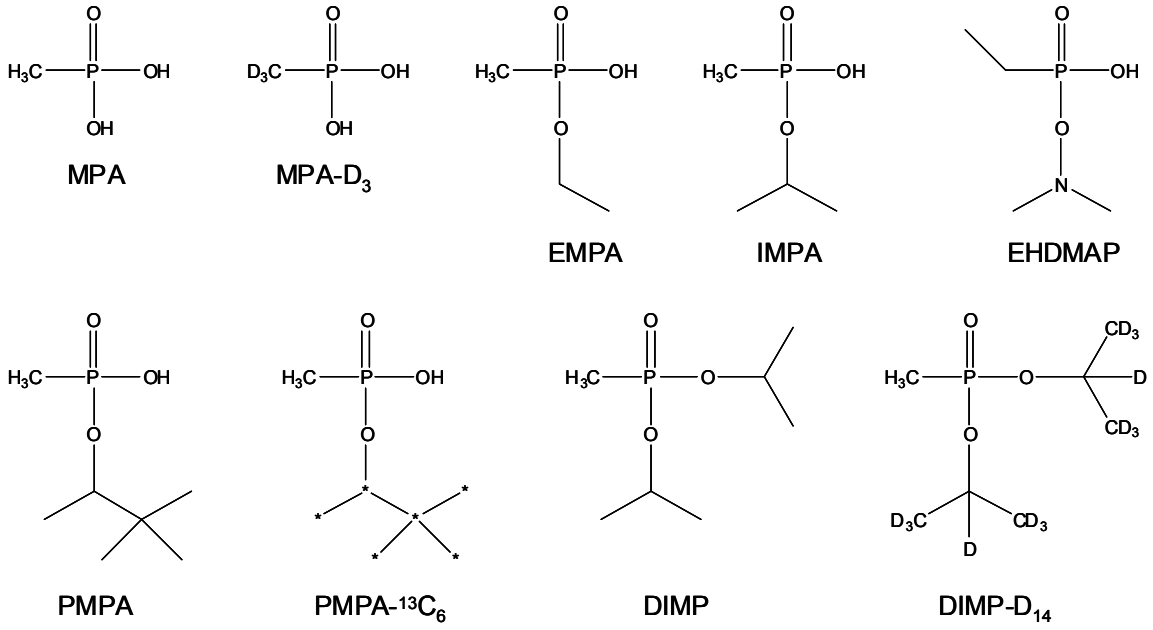


Table 1: Optimization of Tune File Parameters

Parameter	*EPA MS999* Parameters	LLNL Parameters
Capillary voltage, kV	3.5	3.5
Cone voltage, V	Table 2	**Table 2**
Extractor, V	2	2
RF lens, V	0.2	0.2
Source Temp, °C	120	120
Desolvation Temp, °C	300	300
Desolvation gas, L/h	500	500
Cone gas, L/h	25	25
Low mass resolution 1	14.5	14.5
High mass resolution 1	14.5	14.5
Ion energy 1	0.5	0.5
Entrance energy, eV	-1	-1
Collision energy, eV	Table 2	**Table 2**
Exit energy, eV	2	2
Low mass resolution 2	15	15
High mass resolution 2	15	15
Ion energy 2	0.5	**1.5**
Multiplier	650	650
Inter-channel delay, s	0.02	0.02
Inter-scan delay, s	0.3	0.3
Repeats	1	1
Span, Da	0	0
Dwell, s	0.1	0.1

*Entries indicated by bold text were changed from initially described parameters.

Table 2: Parent and product ions for selected analytes and surrogates and optimized cone voltages and collision energies for each

Positive ESI		Analyte						Surrogate		
	Parameter	DIMP	EHDMAP	EMPA	IMPA	MPA	PMPA	MPA-D_3	PMPA-$^{13}C_6$	DIMP-D_{14}
EPA MS999 Parameters	Parent ion, m/z	181.3	154.2	125	139.1	96.9	181.3	99.8	--	195.3
	Product ion, m/z	139.1	126	96.8	96.8	78.7	96.8	81.8	--	147.2
	Cone voltage, V	25	20	25	18	45	15	45	--	25
	Collision energy, eV	6	12	10	9	15	7	15	--	7
	RT (min)	11.0	3.45	3.75	8.90	2.30	10.57	2.35	--	10.97
LLNL Parameters	Parent ion, m/z	181.00	154.18	124.89	138.96	96.74	181.07	99.78	--	195.15
	Product ion, m/z	139.00	126.00	96.78	96.71	78.64	96.71	81.69	--	147.11
	Cone voltage, V	25	20	25	18	**35**	**25**	45	--	**15**
	Collision energy, eV	6	12	10	9	15	7	15	--	7
	RT (min)	11.10	4.05	4.38	8.93	2.87	11.10	2.87	--	11.04

Negative ESI		Analyte						Surrogate		
	Parameter	DIMP	EHDMAP	EMPA	IMPA	MPA	PMPA	MPA-D3	PMPA-$^{13}C_6$	DIMP-D_{14}
EPA MS999 Parameters	Parent ion, m/z	--	152.2	123.1	137.1	94.9	179.2	97.9	185.3	--
	Product ion, m/z	--	78.7	94.8	94.8	78.7	94.8	78.7	94.8	--
	Cone voltage, V	--	30	30	32	35	35	35	35	--
	Collision energy, eV	--	15	12	13	15	18	15	18	--
	RT (min)	--	3.45	3.75	8.90	2.30	10.57	2.35	10.57	--
LLNL Parameters	Parent ion, m/z	--	152.00	122.87	136.95	94.73	179.06	97.77	185.09	--
	Product ion, m/z	--	78.58	94.73	94.73	78.58	94.70	78.58	94.70	--
	Cone voltage, V	--	30	**25**	32	35	**25**	35	35	--
	Collision energy, eV	--	15	**15**	13	15	18	15	18	--
	RT (min)	--	4.00	4.40	8.94	2.85	11.09	2.85	11.09	--

* Values indicated in bold text have been changed from the settings described in *EPA MS999 SOP*

After optimization of tune file parameters, the chromatography was optimized. An Atlantis C18 column (100 x 2.1 mm i.d., 3 µm; Waters Corp., Milford, MA) was utilized for separations of the analyte and surrogate. Rather than using the mobile phase conditions described in *EPA Method MS999* SOP, a binary solvent system was chosen (**Table 3**) with a new flow rate of 200 µL/min rather than the prescribed 300 µL/min. The column compartment was maintained at 30 °C, autosampler at 15 °C, and the column equilibration time was 2 minutes between each sample.

Table 3: Gradient Program for Separation of Phosphonic Acids

Time (min)	Flow (µL/min)	% Acetonitrile	% Water (containing 0.1% formic acid)
0	200	0	100
4	200	0	100
5	200	45	55
9	200	45	55
10	200	95	5
13	200	95	5
14	200	0	100
20	200	0	100

Task 2: Determination of calibration curve data

Analytical calibration standards in water were prepared according to the *EPA Method MS999* SOP from stock solutions prepared in methanol. The concentration of the analytes ranged from 1.25 ppb to 150 ppb (DIMP, DIMP-D_{14}, EHDMAP) and from 12.5 ppb to 1500 ppb (MPA, EMPA, IMPA, PMPA). The surrogates MPA-D_3 and PMPA-$^{13}C_6$ ranged from 6.25 ppb to 750 ppb. The surrogate DIMP-D_{14} ranged in concentration from 1.25 ppb to 150 ppb. The low and high calibration levels that were included in the curve are shown in **Table 4**, along with signal to noise (S/N) data. The limit of detection (LOD) data is provided as well. Briefly, the signal to noise (S/N) was greater than 10 at the Level 1 standard for all analytes by both positive

and negative ion mode ESI, with the exception of EHDMAP (S/N at Level 1 standard was 5.03 by positive ESI and 5.76 by negative ESI).

All calibration curves were quadratic with 1/x weighting; and a minimum of six standards were included in each calibration curve. The two Level 4 standards were analyzed in the middle and end of the sequence list run at the end of the sample list (see **Appendix 1**) and with deviation of less than 10%, with exception of EHDMAP, where deviations were less than 20%. The R^2 values were typically > 0.999 (**Table 4**).

Task 3: Precision and Bias Study

Precision and bias were determined across the calibration ranges by including four replicate samples of reagent water at four different fortification levels (5 or 50 ppb for spike level 1, 10 or 100 ppb for spike level 2, 25 ppb or 250 ppb for spike level 3, and 125 or 1250 ppb for spike level 4) and duplicate samples of surface water at these same fortification levels. Example chromatograms of each analyte in a standard, reagent water, and surface water at spike level 3 (25 ppb or 250 ppb) are shown in **Figure 2**. The results of the precision and bias study are shown in **Tables 5** and **6** (reagent water) and **Tables 7** and **8** (surface water).

Diisopropyl methylphosphonate (DIMP) was analyzed only in positive ion mode ESI. Recoveries of DIMP in reagent water ranged from 94% (at 125 ppb) with a relative standard deviation (RSD) of 5.8% to 120% (at 25 ppb) with RSD of 7%. These values were in very good agreement with the ***Quality Control Acceptance Criteria*** (Table 2 of *EPA MS999 SOP*), where the recoveries of DIMP in reagent water were 75 to 135%. In our analysis of surface water samples, DIMP recoveries ranged from 109% to 126%, which again are in good agreement with Table 2 of *EPA MS999 SOP*.

Ethyl hydrogen dimethylamidophosphonate (EHDMAP) was generally a poor analyte that suffered from poor chromatography. When reagent water samples were analyzed by positive ESI, recoveries ranged from 63% (RSD of 9%) to 73% (RSD of 9%) (**Table 5**). When using negative ESI, the recoveries ranged from non-detectable (for both 5 ppb and 10 ppb spikes) to around 70% recovery for 25 ppb and 125 ppb spikes (**Table 6**). In our analysis of surface water samples, recoveries ranged from 55% to 74% by positive ESI and from non-detectable levels to 57% by negative ESI (**Tables 7** and **8**). While these recoveries are poor, they do agree with the percent recoveries tallied in Table 2 of *EPA MS999 SOP*.

Ethyl methylphosphonic acid (EMPA) was analyzed by both positive and negative ESI. By positive ESI, recoveries of EMPA spiked in reagent water ranged from 100% (RSD of 17.5%) at 50 ppb to 126% (RSD of 7.0%) at 250 ppb (**Table 5**). By negative ESI, these recoveries ranged from 108% to 117% (**Table 6**). When surface water samples were analyzed by positive ESI, recoveries ranged from 113% to 135%, which agreed well with values listed in Table 2 of *EPA MS999 SOP* (**Table 7**). By negative ESI, these recoveries of EMPA in surface water ranged from 88% to 102% (**Table 8**).

When analyzed by positive ESI, isopropyl methylphosphonic acid (IMPA) recoveries in reagent water ranged from 94% (RSD of 22%) to 113% (RSD of 7%) (**Table 5**). When these reagent water samples were analyzed using negative ESI, these recoveries ranged from 91% to 108% (**Table 6**). These recovery ranges by both positive and negative ESI agree well with the *Quality Control Acceptance Criteria* of the *EPA MS999 SOP* (71% to 131% by pos ESI and 70 to 130% by neg ESI). In surface water samples analyzed using positive ESI, recoveries of IMPA ranged from 45% to 57% (**Table 7**). By negative ESI, these recoveries ranged from 43% to

51%. These recoveries are significantly lower than the recovery ranges listed in Table 2 of the *EPA MS999 SOP*.

The recoveries of methylphosphonic acid (MPA) in reagent water, when analyzed by positive ion mode ESI, ranged from 80% when spiked at 50 ppb to 168% when spiked at 1250 ppb (**Table 5**). These recoveries are in good agreement with Table 2 of *EPA MS999 SOP*, though the recovery of 168% is a bit high. When analyzed in negative ion mode ESI, the recoveries for MPA in reagent water ranged from 95% (RSD 6.1%) at 50 ppb to 118% (RSD of 5.6%) at 250 ppb (**Table 6**). These values are in very good agreement with Table 2 of *EPA MS999 SOP*, where the recovery range for MPA is 64 to 124% when analyzed by negative ion mode ESI. Generally, we experienced improved chromatography and lower limits of detection and quantitation when analyzing MPA in negative ion mode (**Table 4**). In surface water, MPA recoveries ranged from non-detectable (at 50 ppb) to 60% (at 100 ppb) when analyzed by positive ESI (**Table 7**). These recoveries are much lower than reported in Table 2 of *EPA MS999 SOP*, where the recoveries in surface water of MPA range from 110 to 170% in matrix spiked samples. When using negative ESI, the recoveries ranged from 9 to 12 % (**Table 8**), and these values are in good agreement with Table 2 of *EPA MS999 SOP*.

Pinacolyl methylphosphonic acid (PMPA), when analyzed by positive ESI, was quantitatively recovered from reagent water and recoveries ranged from 115% to 130% (**Table 5**). When analyzed by negative ESI, these recoveries ranged from 101% to 118% (**Table 6**). These recovery ranges are in good agreement with the value listed in Table 2 of *EPA MS999 SOP* (76 to 136% by pos ESI and 74 to 134% by neg ESI). When PMPA was spiked into surface water and analyzed, recoveries ranged from 114% to 138% by positive ESI (**Table 7**) and 111%

to 120% by negative ESI (**Table 8**). Again, these values are in good agreement with the *Quality Control Acceptance Criteria* of *EPA MS999 SOP*.

The surrogate DIMP-D_{14} was spiked at 25 ppb to all reagent and surface water samples and analyzed only by positive ESI. In reagent water, the recoveries of DIMP-D_{14} ranged from 115% to 130% among the four sample sets. In surface water, the recoveries ranged from 63% to 111%. These values are in good agreement with those previously listed in *EPA MS999 SOP*.

The surrogate MPA-D_3 was spiked at 250 ppb to all reagent and surface water samples and analyzed by both positive and negative ESI. In reagent water, recoveries ranged from 103% to 128% among the four sample sets when analyzed by positive ESI and from 85% to 111% when analyzed by negative ESI (**Tables 5** and **6**). These recoveries are in good agreement with those previously listed in the *EPA MS999 SOP*. When surface water samples were analyzed, the recoveries by positive ESI ranged from 51% to 68% (**Table 7**). These recoveries are significantly lower than those previously listed in the *SOP*. When analyzed by negative ESI, these recoveries among the four sample sets ranged from 12 to 14% (**Table 8**). These low recoveries are in good agreement with the *SOP*.

Finally, the surrogate PMPA-$^{13}C_6$ was added to all surface and reagent water samples at 250 ppb and analyzed only by negative ESI. In reagent water samples, the recoveries of PMPA-$^{13}C_6$ ranged from 86% to 129% among the four sample sets. In surface waters, the recoveries ranged from 100% to 127%. Again these values are in good agreement with the values listed in Table 2 of *EPA MS999 SOP*.

Table 4: Calibration Curve Data for Phosphonic Acids by Positive and Negative Electrospray Ionization

	Analyte	LOD, ppb (S/N)	LOQ, ppb (S/N)	Low standard	High standard	R^2
pos ESI	MPA	50 (4.03)	100 (12.96)	100 ppb	1500 ppb	0.9992
	EMPA	12.5 (4.72)	25 (11.85)	25 ppb	1500 ppb	0.9993
	EHDMAP	1.25 (5.03)	10 (18.55)	10 ppb	150 ppb	0.9996
	IMPA	ND	12.5 (41.04)	12.5 ppb	1500 ppb	0.9995
	PMPA	ND	12.5 (40.06)	12.5 ppb	1500 ppb	0.9992
	DIMP	ND	1.25 (20.23)	1.25 ppb	150 ppb	0.9992
	MPA-D_3	ND	6.25 (10.43)	6.25 ppb	750 ppb	0.9992
	DIMP-D_{14}	ND	1.25 (277.96)	1.25 ppb	150 ppb	0.9970
	PMPA-$^{13}C_6$	--	--	--	--	--

	Analyte	LOD, ppb (S/N)	LOQ, ppb (S/N)	Low standard	High standard	R^2
neg ESI	MPA	ND	12.5 (15.00)	12.5 ppb	1500 ppb	0.9991
	EMPA	--	--	--	--	--
	EHDMAP	10 ppb (5.76)	25 ppb	25 ppb	150 ppb	0.9978
	IMPA	ND	12.5 ppb (19.96)	12.5 ppb	1500 ppb	0.9994
	PMPA	ND	12.5 ppb (52.26)	12.5 ppb	1500 ppb	0.9994
	DIMP	--	--	--	--	--
	MPA-D_3	ND	6.25 ppb (28.59)	6.25 ppb	750 ppb	0.9998
	DIMP-D_{14}	--	--	--	--	--
	PMPA-$^{13}C_6$	ND	6.25 ppb (33.44)	6.25 ppb	750 ppb	0.9996

Table 5: Recoveries of phosphonic acids spiked into four replicates of reagent water and analyzed using positive ion mode electrospray ionization.

Laboratory: **LLNL**
Instrument ID: **Waters Micromass Quattro *micro* API with 2795 HPLC**
Surface Water Description: South Bay Aqueduct water (carries snow-melt from Sierra Nevada mountains to San Diego as a source of drinking water)
Date of Analysis: 10/28/2008

Data Reporting Form 5a. (Phosphonates) Precision and Bias in Reagent Water

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation
Diisopropyl methylphosphonate	5.0	0.0	0.0	4.5	90.0	5.7	114.0	6.0	120.0	6.8	136.0	115	17
Ethyl hydrogen dimethyl amido-phosphate	5.0	0.0	0.0	2.9	58.0	3.3	66.0	3.6	72.0	3.9	78.0	69	12
Ethyl methylphosphonic acid	50.0	0.0	0.0	38.8	77.6	50.0	100.0	51.9	103.8	60.1	120.2	100	17
Isopropyl methylphosphonic acid	50.0	0.0	0.0	33.5	67.0	45.5	91.0	49.9	99.8	58.3	116.6	94	22
Methylphosphonic acid	50.0	0.0	0.0	27.6	55.2	42.6	85.2	44.8	89.6	44.1	88.2	80	21
Pinacolyl methylphosphonic acid	50.0	0.0	0.0	48.3	96.6	60.8	121.6	67.6	135.2	74.4	148.8	126	18
Diisopropyl methylphosphonate-D_{14}	25.0	25.0	22.2	23.3	93.2	24.9	99.6	25.7	102.8	27.4	109.6	101	7
Methylphosphonic acid-D_3	250.0	250.0	253.0	250.8	100.3	286.5	114.6	314.4	125.8	369.4	147.8	122	16
~~Pinacolyl methylphosphonic acid-$^{13}C_6$~~	~~250.0~~	~~250.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0~~	

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
Diisopropyl methylphosphonate	10.0	0.0	0.0	10.5	105.0	10.7	107.0	12.1	121.0	11.0	110.0	111	6
Ethyl hydrogen dimethyl amido-phosphate	10.0	0.0	0.0	6.9	69.0	7.2	72.0	8.5	85.0	5.4	54.0	70	18
Ethyl methylphosphonic acid	100.0	0.0	0.0	108.0	108.0	106.0	106.0	132.5	132.5	85.6	85.6	108	18
Isopropyl methylphosphonic acid	100.0	0.0	0.0	94.9	94.9	93.9	93.9	120.5	120.5	81.1	81.1	98	17
Methylphosphonic acid	100.0	0.0	0.0	112.8	112.8	113.8	113.8	149.7	149.7	75.5	75.5	113	27
Pinacolyl methylphosphonic acid	100.0	0.0	0.0	114.0	114.0	103.7	103.7	135.1	135.1	108.4	108.4	115	12
Diisopropyl methylphosphonate-D_{14}	25.0	25.0	26.4	21.5	86.0	22.9	91.6	24.9	99.6	22.5	90.0	92	6
Methylphosphonic acid-D_3	250.0	250.0	341.8	340.0	136.0	317.6	127.0	379.4	151.8	242.6	97.0	128	18
~~Pinacolyl methylphosphonic acid-$^{13}C_6$~~	~~250.0~~	~~250.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0~~	

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
Diisopropyl methylphosphonate	25.0	0.0	0.0	27.4	109.6	31.0	124.0	32.0	128.0	30.0	120.0	120	7
Ethyl hydrogen dimethyl amido-phosphate	25.0	0.0	0.0	15.7	62.8	19.3	77.2	18.8	75.2	19.1	76.4	73	9
Ethyl methylphosphonic acid	250.0	0.0	0.0	283.4	113.4	326.5	130.6	332.9	133.2	314.6	125.8	126	7
Isopropyl methylphosphonic acid	250.0	0.0	0.0	251.8	100.7	296.1	118.4	296.1	118.4	283.2	113.3	113	7
Methylphosphonic acid	250.0	0.0	0.0	324.9	130.0	372.4	149.0	384.2	153.7	354.4	141.8	144	7
Pinacolyl methylphosphonic acid	250.0	0.0	0.0	293.5	117.4	340.2	136.1	337.1	134.8	326.6	130.6	130	7
Diisopropyl methylphosphonate-D_{14}	25.0	25.0	14.0	21.5	86.0	23.9	95.6	23.7	94.8	22.7	90.8	92	5
Methylphosphonic acid-D_3	250.0	250.0	352.3	255.7	102.3	331.7	132.7	317.4	127.0	344.4	137.8	125	13
~~Pinacolyl methylphosphonic acid-$^{13}C_6$~~	~~250.0~~	~~250.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0~~	

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
Diisopropyl methylphosphonate	125.0	0.0	0.0	112.0	89.6	118.9	95.1	113.3	90.6	127.0	101.6	94	6
Ethyl hydrogen dimethyl amido-phosphate	125.0	0.0	0.0	73.8	59.0	75.8	60.6	74.2	59.4	88.7	71.0	63	9
Ethyl methylphosphonic acid	1250.0	0.0	0.0	1328.4	106.3	1347.0	107.8	1355.6	108.4	1610.5	128.8	113	9
Isopropyl methylphosphonic acid	1250.0	0.0	0.0	1176.9	94.2	1225.0	98.0	1217.4	97.4	1443.7	115.5	101	10
Methylphosphonic acid	1250.0	0.0	0.0	1767.0	141.4	2053.6	164.3	1964.1	157.1	2602.7	208.2	168	17
Pinacolyl methylphosphonic acid	1250.0	0.0	0.0	1281.3	102.5	1327.3	106.2	1248.4	99.9	1469.0	117.5	107	7
Diisopropyl methylphosphonate-D_{14}	25.0	25.0	30.6	25.1	100.4	11.7	46.8	13.7	54.8	12.3	49.2	63	40
Methylphosphonic acid-D_3	250.0	250.0	289.4	240.6	96.2	246.2	98.5	251.6	100.6	291.8	116.7	103	9
~~Pinacolyl methylphosphonic acid-$^{13}C_6$~~	~~250.0~~	~~250.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0~~	

Table 6: Recoveries of phosphonic acids spiked into four replicates of reagent water and analyzed using negative ion mode electrospray ionization.

Laboratory: **LLNL**
Instrument ID: **Waters Micromass Quattro *micro* API with 2795 HPLC**
Surface Water Description: South Bay Aqueduct water (carries snow-melt from Sierra Nevada mountains to San Diego as a source of drinking water)
Date of Analysis: 10/28/2008

Data Reporting Form 5a. (Phosphonates) Precision and Bias in Reagent Water

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation
~~Diisopropyl methylphosphonate~~	~~5.0~~	~~0.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0.0~~	
Ethyl hydrogen dimethyl amido-phosphate	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Ethyl methylphosphonic acid	50.0	0.0	0.0	51.1	102.2	62.5	125.0	66.6	133.2	52.7	105.4	116.5	12.9
Isopropyl methylphosphonic acid	50.0	0.0	0.0	32.3	64.6	45.1	90.2	46.5	93.0	57.0	114.0	90.5	22.4
Methylphosphonic acid	50.0	0.0	0.0	44.2	88.4	46.2	92.4	49.9	99.8	50.1	100.2	95.2	6.1
Pinacolyl methylphosphonic acid	50.0	0.0	0.0	44.1	88.2	52.7	105.4	53.5	107.0	62.2	124.4	106.3	13.9
~~Diisopropyl methylphosphonate-D_{14}~~	~~250.~~	~~25.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0.0~~	
Methylphosphonic acid-D_3	250.0	250.0	251.0	235.6	94.2	270.5	108.2	287.9	115.2	303.3	121.3	109.7	10.6
Pinacolyl methylphosphonic acid-$^{13}C_6$	250.0	250.0	299.7	261.8	104.7	320.8	128.3	318.0	127.2	385.2	154.1	128.6	15.7

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
~~Diisopropyl methylphosphonate~~	~~10.0~~	~~0.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0.0~~	
Ethyl hydrogen dimethyl amido-phosphate	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Ethyl methylphosphonic acid	100.0	0.0	0.0	87.7	87.7	116.7	116.7	121.9	121.9	104.6	104.6	107.7	14.1
Isopropyl methylphosphonic acid	100.0	0.0	0.0	92.3	92.3	87.1	87.1	115.7	115.7	72.2	72.2	91.8	19.7
Methylphosphonic acid	100.0	0.0	0.0	99.4	99.4	97.8	97.8	123.0	123.0	93.6	93.6	103.5	12.8
Pinacolyl methylphosphonic acid	100.0	0.0	0.0	96.9	96.9	91.0	91.0	117.4	117.4	98.2	98.2	100.9	11.4
~~Diisopropyl methylphosphonate-D_{14}~~	~~250.~~	~~25.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0.0~~	
Methylphosphonic acid-D_3	250.0	250.0	293.3	304.4	121.8	264.0	105.6	293.4	117.4	228.0	91.2	109.0	12.5
Pinacolyl methylphosphonic acid-$^{13}C_6$	250.0	250.0	322.6	316.0	126.4	263.3	105.3	314.6	125.8	266.9	106.8	116.1	10.0

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
~~Diisopropyl methylphosphonate~~	~~250.~~	~~0.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0.0~~	
Ethyl hydrogen dimethyl amido-phosphate	25.0	0.0	0.0	17.7	70.8	19.0	76.0	18.9	75.6	17.0	68.0	72.6	5.3
Ethyl methylphosphonic acid	250.0	0.0	0.0	263.9	105.6	301.3	120.5	298.6	119.4	306.6	122.6	117.0	6.6
Isopropyl methylphosphonic acid	250.0	0.0	0.0	229.2	91.7	289.8	115.9	286.7	114.7	278.3	111.3	108.4	10.4
Methylphosphonic acid	250.0	0.0	0.0	272.3	108.9	304.8	121.9	309.4	123.8	291.9	116.8	117.8	5.6
Pinacolyl methylphosphonic acid	250.0	0.0	0.0	269.8	107.9	309.4	123.8	303.2	121.3	297.5	119.0	118.0	5.9
~~Diisopropyl methylphosphonate-D_{14}~~	~~250.~~	~~25.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0.0~~	
Methylphosphonic acid-D_3	250.0	250.0	281.0	237.4	95.0	285.1	114.0	294.0	117.6	293.3	117.3	111.0	9.7
Pinacolyl methylphosphonic acid-$^{13}C_6$	250.0	250.0	317.9	241.9	96.8	304.7	121.9	300.3	120.1	282.6	113.0	113.0	10.1

		Reagent Water Blank		Sample 1		Sample 2		Sample 3		Sample 4		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Standard Deviation (RSD)
~~Diisopropyl methylphosphonate~~	~~125.0~~	~~0.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0.0~~	
Ethyl hydrogen dimethyl amido-phosphate	125.0	0.0	0.0	85.7	68.6	80.5	64.4	88.3	70.6	89.3	71.4	68.8	4.6
Ethyl methylphosphonic acid	1250.0	0.0	0.0	1349.0	107.9	1471.2	117.7	1443.0	115.4	1397.5	111.8	113.2	3.8
Isopropyl methylphosphonic acid	1250.0	0.0	0.0	1062.7	85.0	1133.6	90.7	1066.1	85.3	1287.4	103.0	91.0	9.2
Methylphosphonic acid	1250.0	0.0	0.0	1205.7	96.5	1292.9	103.4	1295.0	103.6	1471.9	117.8	105.3	8.5
Pinacolyl methylphosphonic acid	1250.0	0.0	0.0	1213.3	97.1	1249.7	100.0	1227.2	98.2	1420.1	113.6	102.2	7.5
~~Diisopropyl methylphosphonate-D_{14}~~	~~250.~~	~~25.0~~			~~0.0~~		~~0.0~~		~~0.0~~		~~0.0~~	~~0.0~~	
Methylphosphonic acid-D_3	250.0	250.0	266.1	191.5	76.6	205.9	82.4	211.1	84.4	236.7	94.7	84.5	8.9
Pinacolyl methylphosphonic acid-$^{13}C_6$	250.0	250.0	270.3	200.4	80.2	206.8	82.7	201.5	80.6	247.4	99.0	85.6	10.5

Table 7: Recoveries of phosphonic acids spiked into duplicate samples of surface water and analyzed using positive ion mode electrospray ionization.

Laboratory: **LLNL**
Instrument ID: **Waters Micromass Quattro *micro* API with 2795 HPLC**
Surface Water Description: South Bay Aqueduct water (carries snow-melt from Sierra Nevada mountains to San Diego as a source of drin
Date of Analyses: 10/28/2008

Data Reporting Form 5b: (Phosphonates) Precision and Bias in Local Surface Water

		Surface Water Blank		Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
Diisopropyl methylphosphonate	5.0	0.0	0.0	6.5	130.0	6.1	122.0	126.0	6.3
Ethyl hydrogen dimethyl amido-phosphate	5.0	0.0	0.0	3.7	74.0	3.7	74.0	74.0	0.0
Ethyl methylphosphonic acid	50.0	0.0	0.0	66.0	132.0	68.8	137.6	134.8	-4.2
Isopropyl methylphosphonic acid	50.0	0.0	0.0	28.4	56.8	28.5	57.0	56.9	-0.4
Methylphosphonic acid	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Pinacolyl methylphosphonic acid	50.0	0.0	0.0	69.4	138.8	68.7	137.4	138.1	1.0
Diisopropyl methylphosphonate-D_{14}	25.0	25.0	28.1	25.7	102.8	25.8	103.2	103.0	-0.4
Methylphosphonic acid-D_3	250.0	250.0	166.1	172.2	68.9	168.9	67.6	68.2	1.9
~~Pinacolyl methylphosphonic acid-$^{13}C_6$~~	~~250.0~~	~~250.0~~			~~0.0~~		~~0.0~~	~~0.0~~	

		Surface Water Blank		Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
Diisopropyl methylphosphonate	10.0	0.0	0.0	13.1	131.0	10.9	109.0	120.0	18.3
Ethyl hydrogen dimethyl amido-phosphate	10.0	0.0	0.0	7.8	78.0	5.9	59.0	68.5	27.7
Ethyl methylphosphonic acid	100.0	0.0	0.0	140.2	140.2	121.4	121.4	130.8	14.4
Isopropyl methylphosphonic acid	100.0	0.0	0.0	49.2	49.2	40.4	40.4	44.8	19.6
Methylphosphonic acid	100.0	0.0	0.0	65.8	65.8	54.4	54.4	60.1	19.0
Pinacolyl methylphosphonic acid	100.0	0.0	0.0	144.0	144.0	115.8	115.8	129.9	21.7
Diisopropyl methylphosphonate-D_{14}	25.0	25.0	29.4	29.5	118.0	26.1	104.4	111.2	12.2
Methylphosphonic acid-D_3	250.0	250.0	153.0	177.2	70.9	152.3	60.9	65.9	15.1
~~Pinacolyl methylphosphonic acid-$^{13}C_6$~~	~~250.0~~	~~250.0~~			~~0.0~~		~~0.0~~	~~0.0~~	

		Surface Water Blank		Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
Diisopropyl methylphosphonate	25.0	0.0	0.0	25.4	101.6	31.3	125.2	113.4	-20.8
Ethyl hydrogen dimethyl amido-phosphate	25.0	0.0	0.0	11.8	47.2	15.6	62.4	54.8	-27.7
Ethyl methylphosphonic acid	250.0	0.0	0.0	246.4	98.6	317.4	127.0	112.8	-25.2
Isopropyl methylphosphonic acid	250.0	0.0	0.0	100.0	40.0	127.9	51.2	45.6	-24.5
Methylphosphonic acid	250.0	0.0	0.0	123.8	49.5	146.8	58.7	54.1	-17.0
Pinacolyl methylphosphonic acid	250.0	0.0	0.0	255.4	102.2	321.8	128.7	115.4	-23.0
Diisopropyl methylphosphonate-D_{14}	25.0	25.0	28.2	18.2	72.8	23.5	94.0	83.4	-25.4
Methylphosphonic acid-D_3	250.0	250.0	135.7	143.8	57.5	152.2	60.9	59.2	-5.7
~~Pinacolyl methylphosphonic acid-$^{13}C_6$~~	~~250.0~~	~~250.0~~			~~0.0~~		~~0.0~~	~~0.0~~	

		Surface Water Blank		Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
Diisopropyl methylphosphonate	125.0	0.0	0.0	144.7	115.8	128.7	103.0	109.4	11.7
Ethyl hydrogen dimethyl amido-phosphate	125.0	0.0	0.0	79.6	63.7	67.9	54.3	59.0	15.9
Ethyl methylphosphonic acid	1250.0	0.0	0.0	1562.9	125.0	1369.1	109.5	117.3	13.2
Isopropyl methylphosphonic acid	1250.0	0.0	0.0	764.5	61.2	588.8	47.1	54.1	26.0
Methylphosphonic acid	1250.0	0.0	0.0	668.1	53.4	648.8	51.9	52.7	2.9
Pinacolyl methylphosphonic acid	1250.0	0.0	0.0	1497.5	119.8	1361.4	108.9	114.4	9.5
Diisopropyl methylphosphonate-D_{14}	25.0	25.0	4.4	16.6	66.4	14.7	58.8	62.6	12.1
Methylphosphonic acid-D_3	250.0	250.0	17.8	133.0	53.2	122.5	49.0	51.1	8.2
~~Pinacolyl methylphosphonic acid-$^{13}C_6$~~	~~250.0~~	~~250.0~~			~~0.0~~		~~0.0~~	~~0.0~~	

Table 8: Recoveries of phosphonic acids spiked into duplicate samples of surface water and analyzed using negative ion mode electrospray ionization.

Laboratory: **LLNL**
Instrument ID: **Waters Micromass Quattro *micro* API with 2795 HPLC**
Surface Water Description: South Bay Aqueduct water (carries snow-melt from Sierra Nevada mountains to San Diego as a source of drin
Date of Analyses: 10/28/2008

Data Reporting Form 5b: (Phosphonates) Precision and Bias in Local Surface Water

		Surface Water Blank		Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
~~Diisopropyl methylphosphonate~~	~~5.0~~	~~0.0~~			~~0.0~~		~~0.0~~	~~0.0~~	
Ethyl hydrogen dimethyl amido-phosphate	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Ethyl methylphosphonic acid	50.0	0.0	0.0	46.4	92.8	44.0	88.0	90.4	5.3
Isopropyl methylphosphonic acid	50.0	0.0	0.0	26.6	53.2	24.5	49.0	51.1	8.2
Methylphosphonic acid	50.0	0.0	0.0	4.2	8.4	4.3	8.6	8.5	-2.4
Pinacolyl methylphosphonic acid	50.0	0.0	0.0	55.3	110.6	54.6	109.2	109.9	1.3
~~Diisopropyl methylphosphonate-D_{14}~~	~~25.0~~	~~25.0~~			~~0.0~~		~~0.0~~	~~0.0~~	
Methylphosphonic acid-D_3	250.0	250.0	27.5	34.8	13.9	33.8	13.5	13.7	2.9
Pinacolyl methylphosphonic acid-$^{13}C_6$	250.0	250.0	315.0	311.5	124.6	323.3	129.3	127.0	-3.7

		Surface Water Blank		Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
~~Diisopropyl methylphosphonate~~	~~10.0~~	~~0.0~~			~~0.0~~		~~0.0~~	~~0.0~~	
Ethyl hydrogen dimethyl amido-phosphate	10.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Ethyl methylphosphonic acid	100.0	0.0	0.0	85.2	85.2	90.1	90.1	87.7	-5.6
Isopropyl methylphosphonic acid	100.0	0.0	0.0	52.8	52.8	37.9	37.9	45.4	32.9
Methylphosphonic acid	100.0	0.0	0.0	9.7	9.7	7.7	7.7	8.7	23.0
Pinacolyl methylphosphonic acid	100.0	0.0	0.0	121.5	121.5	100.1	100.1	110.8	19.3
~~Diisopropyl methylphosphonate-D_{14}~~	~~25.0~~	~~25.0~~			~~0.0~~		~~0.0~~	~~0.0~~	
Methylphosphonic acid-D_3	250.0	250.0	28.0	32.8	13.1	26.8	10.7	11.9	20.1
Pinacolyl methylphosphonic acid-$^{13}C_6$	250.0	250.0	339.5	336.6	134.6	284.2	113.7	124.2	16.9

		Surface Water Blank		Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
~~Diisopropyl methylphosphonate~~	~~25.0~~	~~0.0~~			~~0.0~~		~~0.0~~	~~0.0~~	
Ethyl hydrogen dimethyl amido-phosphate	25.0	0.0	0.0	8.9	35.6	15.3	61.2	48.4	-52.9
Ethyl methylphosphonic acid	250.0	0.0	0.0	184.9	74.0	252.4	101.0	87.5	-30.9
Isopropyl methylphosphonic acid	250.0	0.0	0.0	89.5	35.8	124.9	50.0	42.9	-33.0
Methylphosphonic acid	250.0	0.0	0.0	23.0	9.2	32.8	13.1	11.2	-35.1
Pinacolyl methylphosphonic acid	250.0	0.0	0.0	244.9	98.0	301.0	120.4	109.2	-20.6
~~Diisopropyl methylphosphonate-D_{14}~~	~~25.0~~	~~25.0~~			~~0.0~~		~~0.0~~	~~0.0~~	
Methylphosphonic acid-D_3	250.0	250.0	26.7	29.3	11.7	30.9	12.4	12.0	-5.3
Pinacolyl methylphosphonic acid-$^{13}C_6$	250.0	250.0	293.9	283.2	113.3	318.7	127.5	120.4	-11.8

		Surface Water Blank		Sample 1		Sample 2		Recovery	
Analyte/Surrogate	Sample Spike Concentration (PPB)	Blank Spike Concentration (PPB)	Recovered (PPB)	Recovered (PPB)	Percent Recovery	Recovered (PPB)	Percent Recovery	Mean %	Relative Percent Difference (RPD)
~~Diisopropyl methylphosphonate~~	~~125.0~~	~~0.0~~			~~0.0~~		~~0.0~~	~~0.0~~	
Ethyl hydrogen dimethyl amido-phosphate	125.0	0.0	0.0	80.8	64.6	60.7	48.6	56.6	28.4
Ethyl methylphosphonic acid	1250.0	0.0	0.0	1234.8	98.8	1308.1	104.6	101.7	-5.8
Isopropyl methylphosphonic acid	1250.0	0.0	0.0	719.4	57.6	505.5	40.4	49.0	34.9
Methylphosphonic acid	1250.0	0.0	0.0	160.2	12.8	149.7	12.0	12.4	6.8
Pinacolyl methylphosphonic acid	1250.0	0.0	0.0	1610.9	128.9	1399.2	111.9	120.4	14.1
~~Diisopropyl methylphosphonate-D_{14}~~	~~25.0~~	~~25.0~~			~~0.0~~		~~0.0~~	~~0.0~~	
Methylphosphonic acid-D_3	250.0	250.0	12.5	33.5	13.4	31.4	12.6	13.0	6.5
Pinacolyl methylphosphonic acid-$^{13}C_6$	250.0	250.0	53.8	264.9	106.0	235.8	94.3	100.1	11.6

Appendix 1: Sequence List

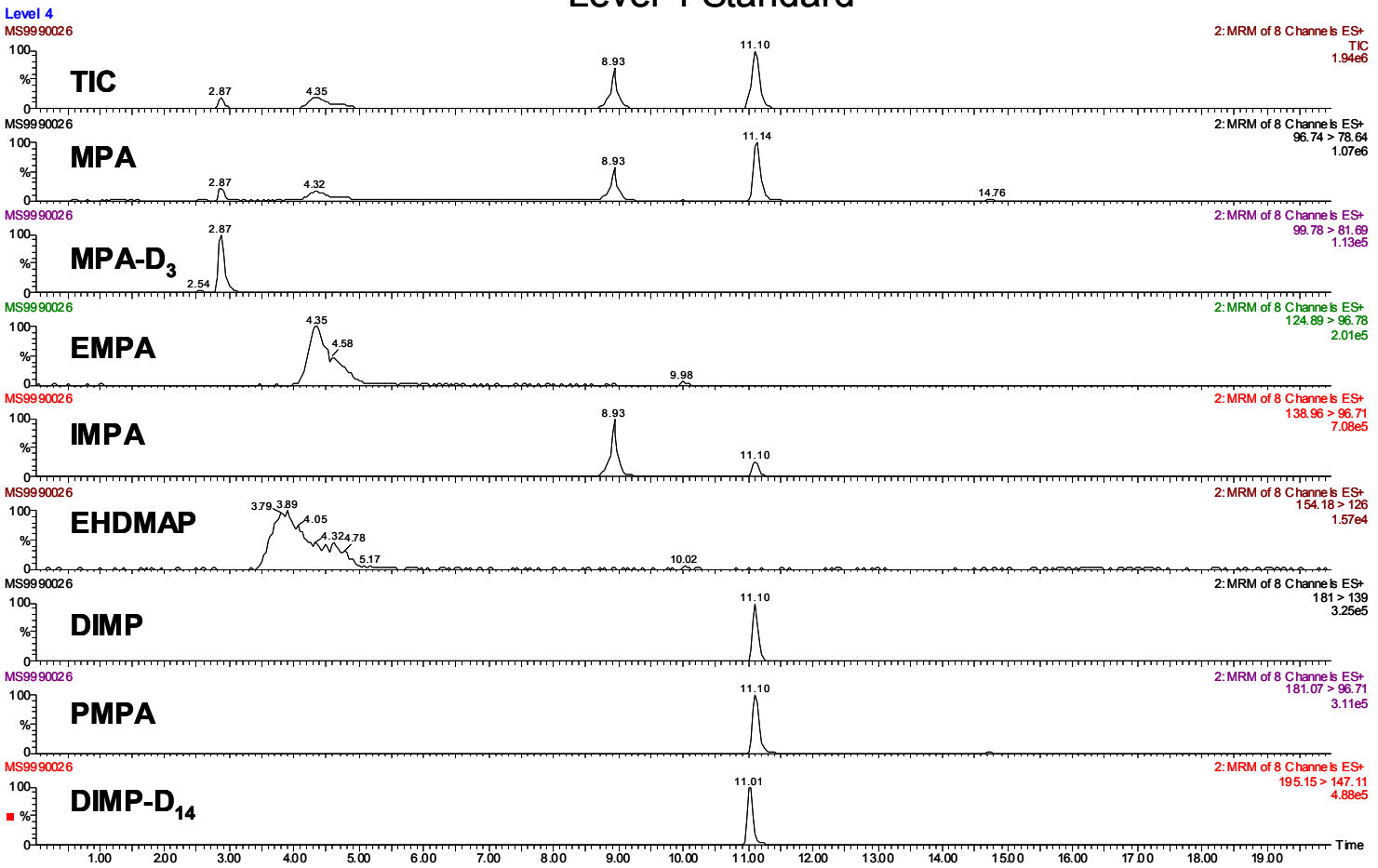
MassLynx - Sample List

Sample List: C:\MassLynx\MS666.PRO\SampleDB\102808.SPL
Printed: Thursday, October 30, 2008 10:19:03

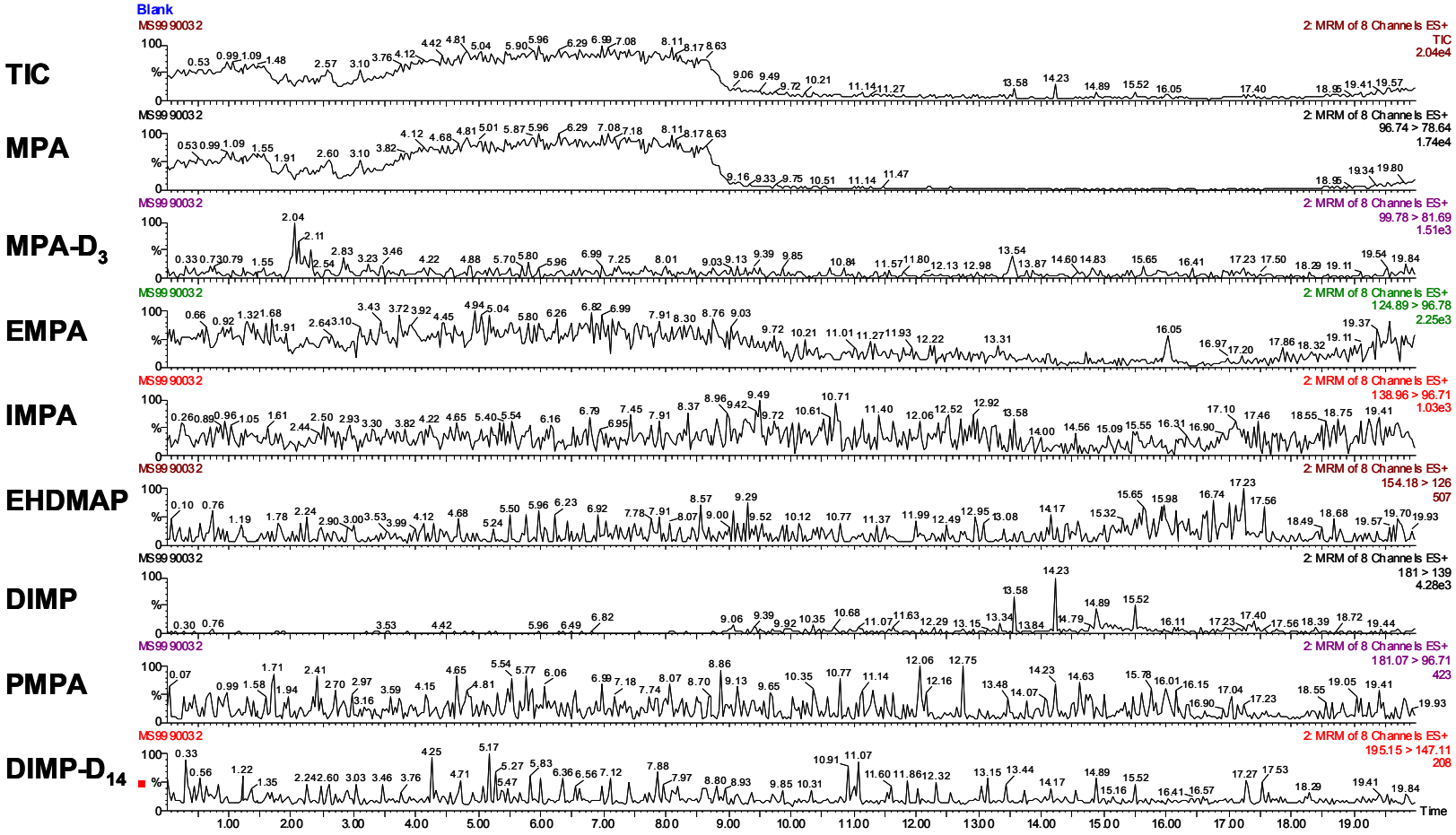
	File Name	File Text	MS File	Inlet File	Bottle	Inject Volume	Control	Sample Type	Conc A
1	MS9990021	Level 7; EPA-STDS-3-135-1	MS999	MS999	3:1	50.000000	---	---	---
2	MS9990022	Level 7; EPA-STDS-3-135-1	MS999	MS999	3:1	50.000000	---	---	---
3	MS9990023	Level 7; EPA-STDS-3-135-1	MS999	MS999	3:1	50.000000	---	Standard	150
4	MS9990024	Level 6; EPA-STDS-3-137-1	MS999	MS999	3:2	50.000000	---	Standard	100
5	MS9990025	Level 5; EPA-STDS-3-138-1	MS999	MS999	3:3	50.000000	---	Standard	50
6	MS9990026	Level 4; EPA-STDS-3-139-1	MS999	MS999	3:4	50.000000	---	Standard	35
7	MS9990027	Level 3; EPA-STDS-3-140-1	MS999	MS999	3:5	50.000000	---	Standard	20
8	MS9990028	Level 2; EPA-STDS-3-141-1	MS999	MS999	3:6	50.000000	---	Standard	10
9	MS9990029	Level 1; EPA-STDS-3-142-1	MS999	MS999	3:7	50.000000	---	Standard	5
10	MS9990030	Level 1/2; EPA-STDS-3-143-1	MS999	MS999	3:8	50.000000	---	Standard	2.5
11	MS9990031	Level 1/4; EPA-STDS-3-144-1	MS999	MS999	3:9	50.000000	---	Standard	1.25
12	MS9990032	Blank	MS999	MS999	3:10	50.000000	---	Blank	---
13	MS9990033	SWB4	MS999	MS999	3:11	50.000000	---	Analyte	---
14	MS9990034	SW + Spike level 4, A	MS999	MS999	3:12	50.000000	---	Analyte	---
15	MS9990035	SW + Spike level 4, B	MS999	MS999	3:13	50.000000	---	Analyte	---
16	MS9990036	SWB3	MS999	MS999	3:14	50.000000	---	Analyte	---
17	MS9990037	SW + Spike level 3, A	MS999	MS999	3:15	50.000000	---	Analyte	---
18	MS9990038	SW + Spike level 3, B	MS999	MS999	3:16	50.000000	---	Analyte	---
19	MS9990039	SWB2	MS999	MS999	3:17	50.000000	---	Analyte	---
20	MS9990040	SW + Spike level 2, A	MS999	MS999	3:18	50.000000	---	Analyte	---
21	MS9990041	SW + Spike level 2, B	MS999	MS999	3:19	50.000000	---	Analyte	---
22	MS9990042	SWB1	MS999	MS999	3:20	50.000000	---	Analyte	---
23	MS9990043	SW + Spike level 1, A	MS999	MS999	3:21	50.000000	---	Analyte	---
24	MS9990044	SW + Spike level 1, B	MS999	MS999	3:22	50.000000	---	Analyte	---
25	MS9990045	Blank	MS999	MS999	3:10	50.000000	---	Analyte	---
26	MS9990046	Level 4; EPA-STDS-3-139-1	MS999	MS999	3:4	50.000000	---	Standard	35
27	MS9990047	RWB4	MS999	MS999	3:23	50.000000	---	Analyte	---
28	MS9990048	RW + Spike level 4, A	MS999	MS999	3:24	50.000000	---	Analyte	---
29	MS9990049	RW + Spike level 4, B	MS999	MS999	3:25	50.000000	---	Analyte	---
30	MS9990050	RW + Spike level 4, C	MS999	MS999	3:26	50.000000	---	Analyte	---
31	MS9990051	RW + Spike level 4, D	MS999	MS999	3:27	50.000000	---	Analyte	---
32	MS9990052	RWB3	MS999	MS999	3:28	50.000000	---	Analyte	---
33	MS9990053	RW + Spike level 3, A	MS999	MS999	3:29	50.000000	---	Analyte	---
34	MS9990054	RW + Spike level 3, B	MS999	MS999	3:30	50.000000	---	Analyte	---
35	MS9990055	RW + Spike level 3, C	MS999	MS999	3:31	50.000000	---	Analyte	---
36	MS9990056	RW + Spike level 3, D	MS999	MS999	3:32	50.000000	---	Analyte	---
37	MS9990057	RWB2	MS999	MS999	3:33	50.000000	---	Analyte	---
38	MS9990058	RW + Spike level 2, A	MS999	MS999	3:34	50.000000	---	Analyte	---
39	MS9990059	RW + Spike level 2, B	MS999	MS999	3:35	50.000000	---	Analyte	---
40	MS9990060	RW + Spike level 2, C	MS999	MS999	3:36	50.000000	---	Analyte	---
41	MS9990061	RW + Spike level 2, D	MS999	MS999	3:37	50.000000	---	Analyte	---
42	MS9990062	RWB1	MS999	MS999	3:38	50.000000	---	Analyte	---
43	MS9990063	RW + Spike level 1, A	MS999	MS999	3:39	50.000000	---	Analyte	---
44	MS9990064	RW + Spike level 1, B	MS999	MS999	3:40	50.000000	---	Analyte	---
45	MS9990065	RW + Spike level 1, C	MS999	MS999	3:41	50.000000	---	Analyte	---
46	MS9990066	RW + Spike level 1, D	MS999	MS999	3:42	50.000000	---	Analyte	---
47	MS9990067	Blank	MS999	MS999	3:10	50.000000	---	Analyte	---
48	MS9990068	Level 4; EPA-STDS-3-139-1	MS999	MS999	3:4	50.000000	---	Standard	35
49	MS9990069	Blank	MS999	MS999	3:10	50.000000	---	Analyte	---
50	MS9990070	IS Mix	MS999	MS999	3:43	50.000000	---	Analyte	---

Appendix 2: Example chromatograms from precision/bias study

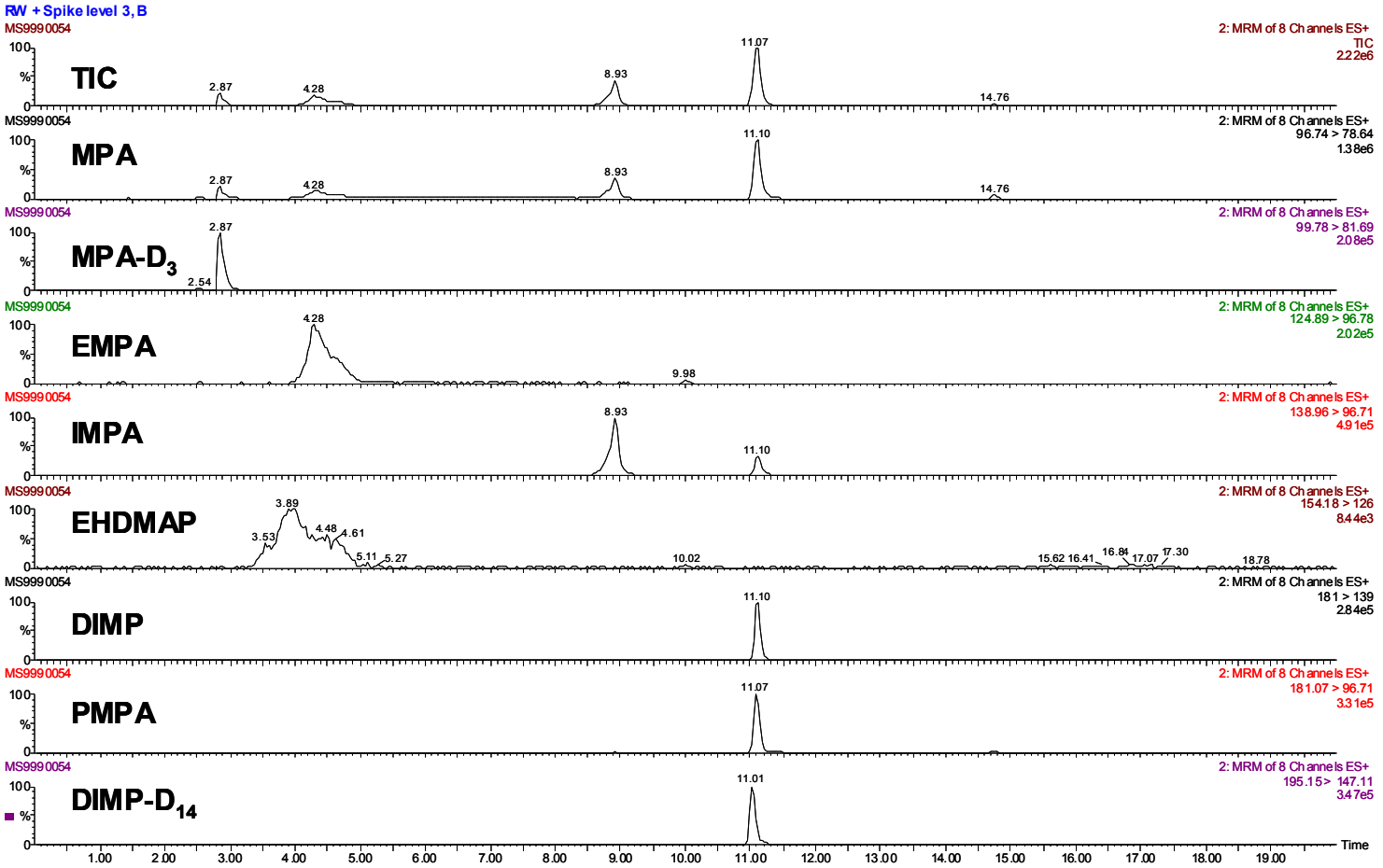
Chromatograms of Phosphonic Acids by Positive Ion Mode Electrospray Ionization: Level 4 Standard



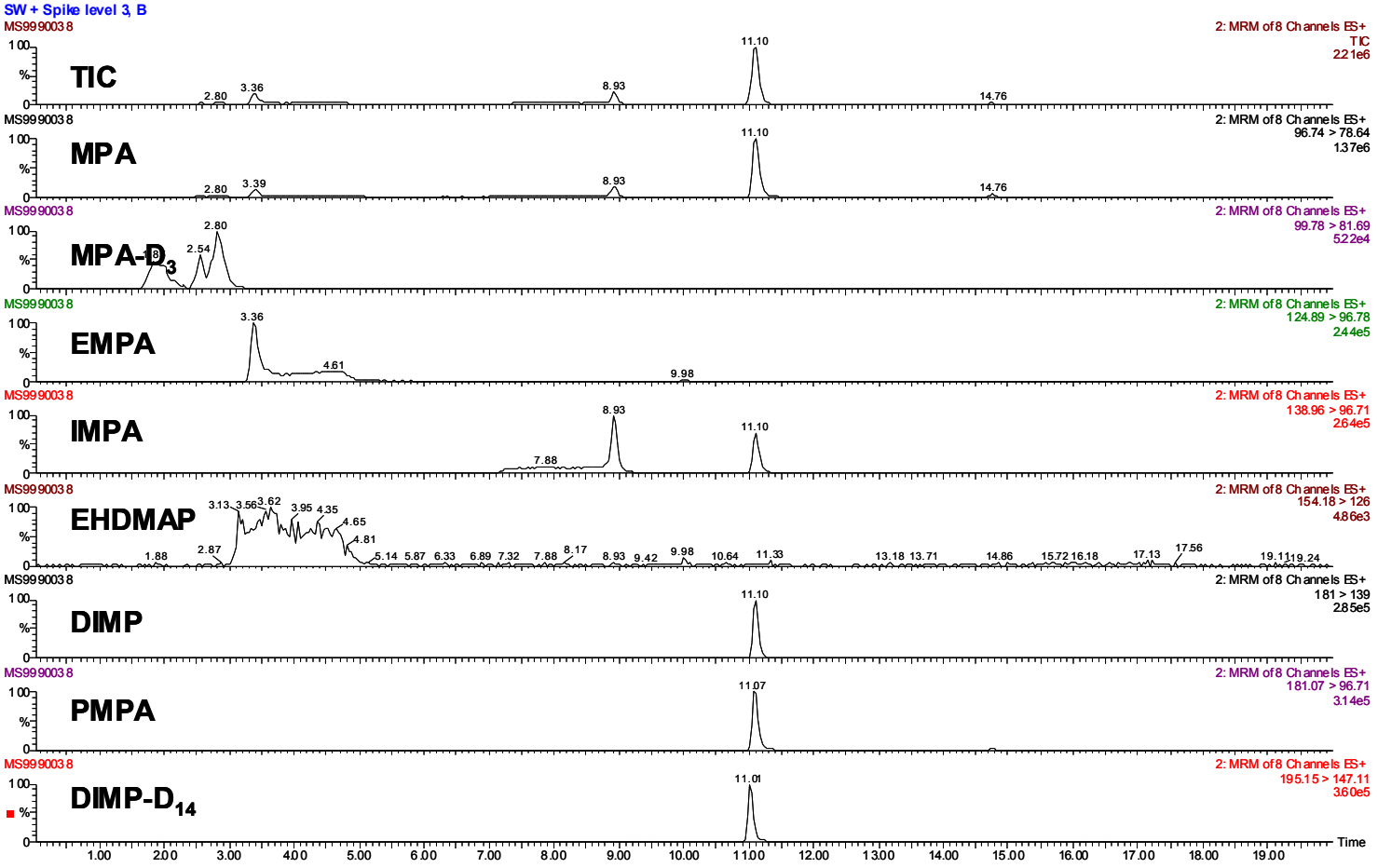
Chromatograms of Phosphonic Acids by Positive Ion Mode Electrospray Ionization: Reagent Blank



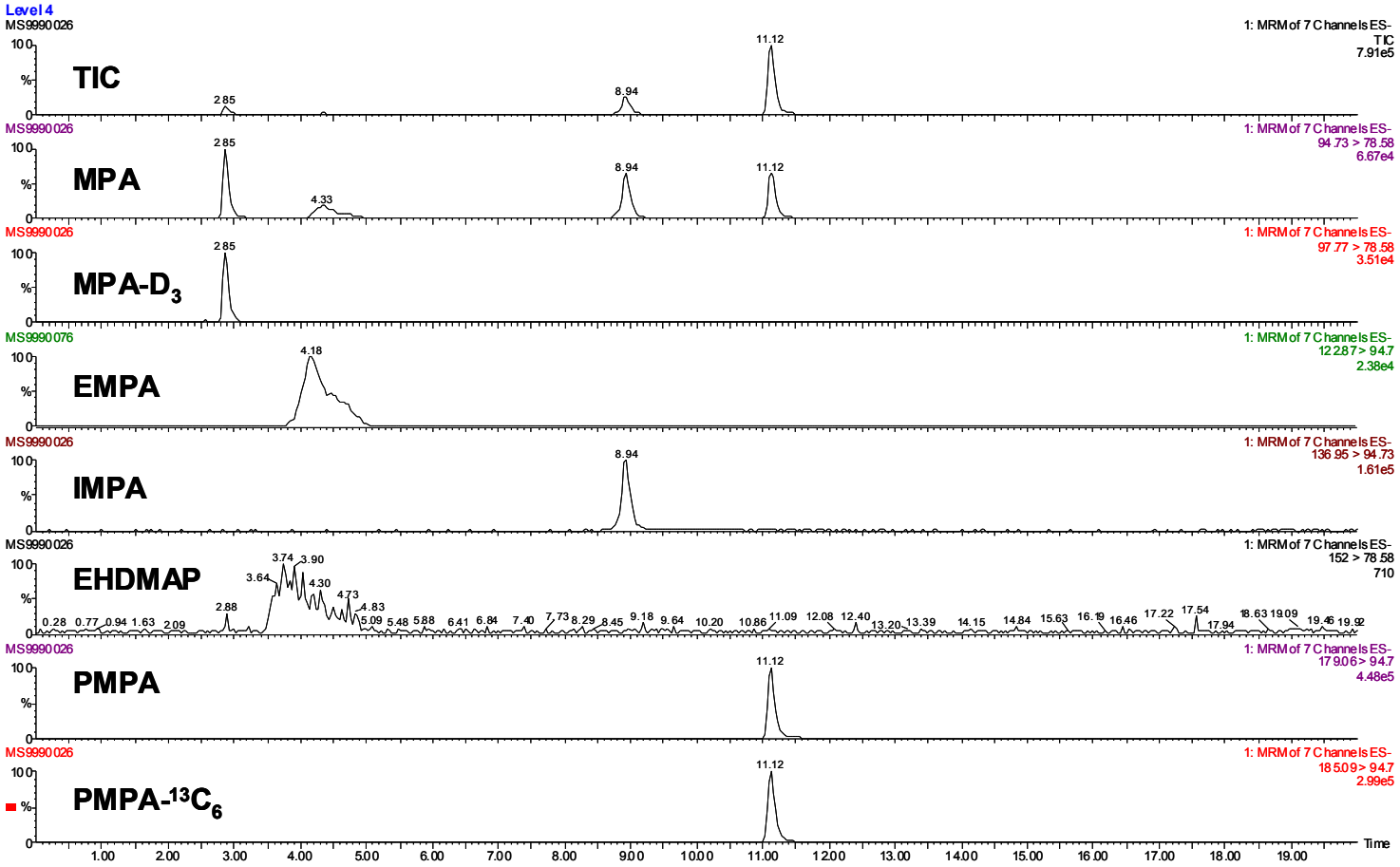
Chromatograms of Phosphonic Acids by Positive Ion Mode Electrospray Ionization: Spike Level 3 (25 ppb or 250 ppb) in Reagent Water



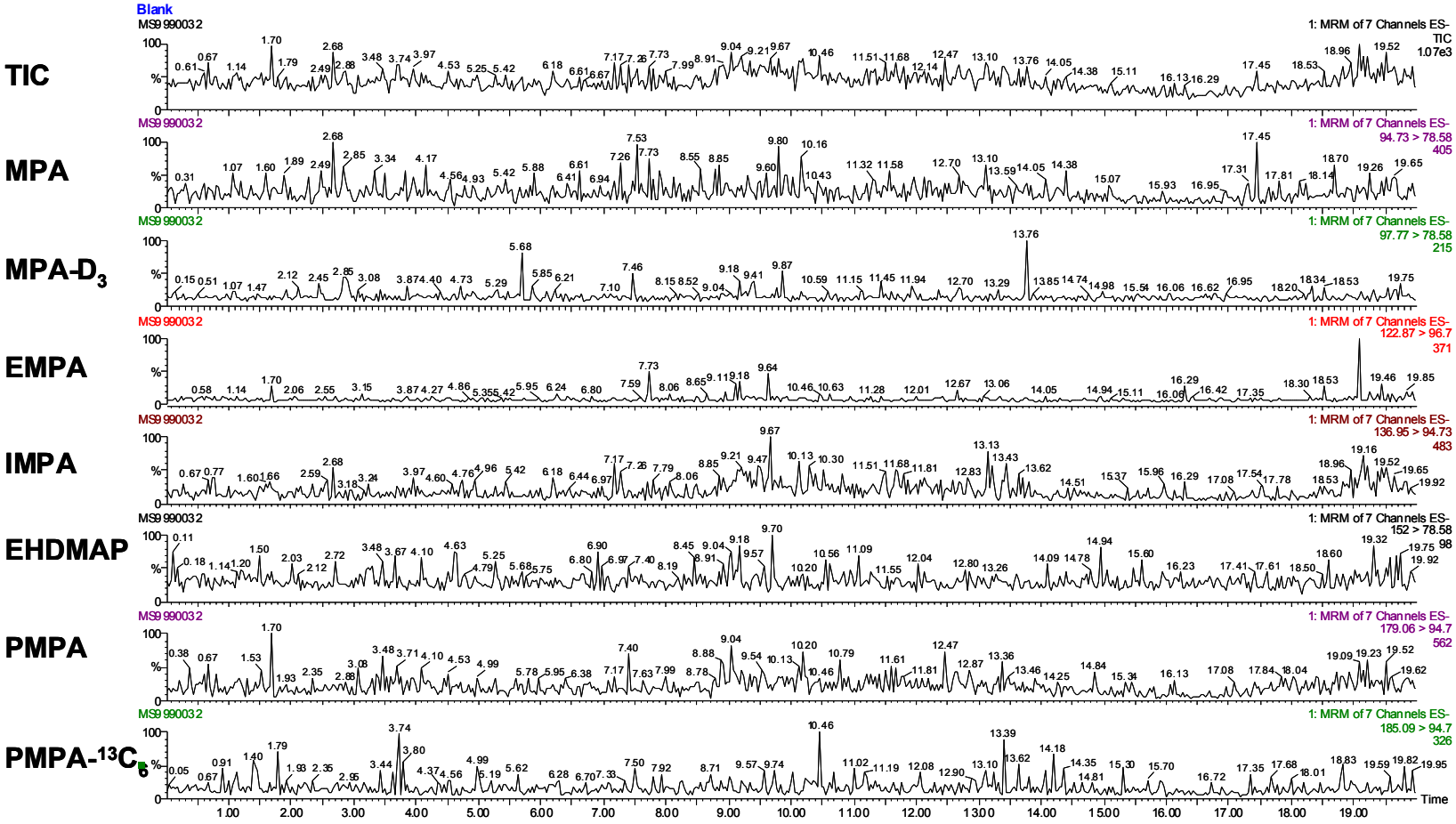
Chromatograms of Phosphonic Acids by Positive Ion Mode Electrospray Ionization: Spike Level 3 (25 ppb or 250 ppb) in Surface Water



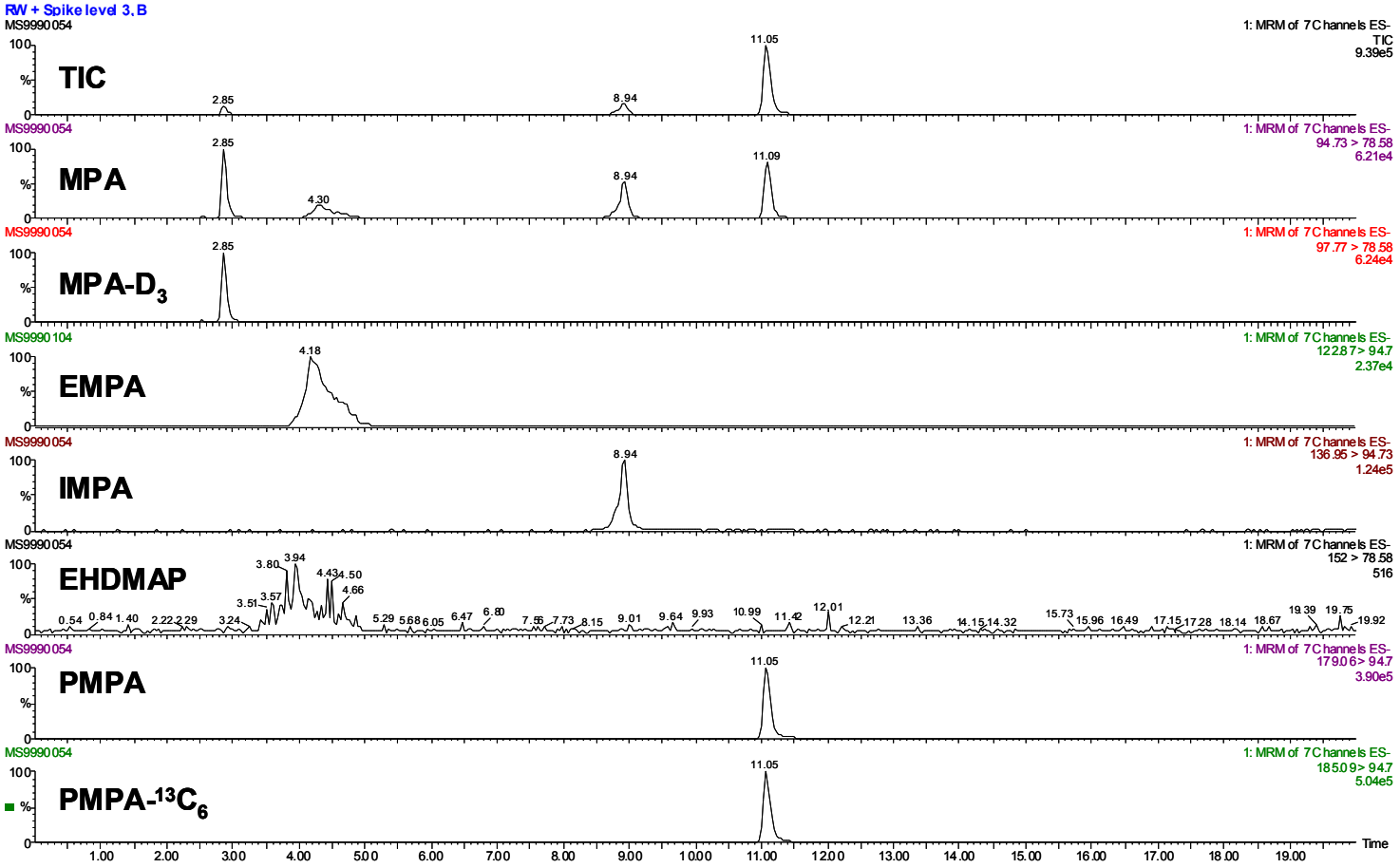
Chromatograms of Phosphonic Acids by Negative Ion Mode Electrospray Ionization: Level 4 Standard



Chromatograms of Phosphonic Acids by Negative Ion Mode Electrospray Ionization: Reagent Blank



Chromatograms of Phosphonic Acids by Negative Ion Mode Electrospray Ionization: Spike Level 3 (25 ppb or 250 ppb) in Reagent Water



Chromatograms of Phosphonic Acids by Negative Ion Mode Electrospray Ionization: Spike Level 3 (25 ppb or 250 ppb) in Surface Water