Analysis of Acid Herbicides in Drinking and Surface Water Using On-line SPE-LC-MS/MS

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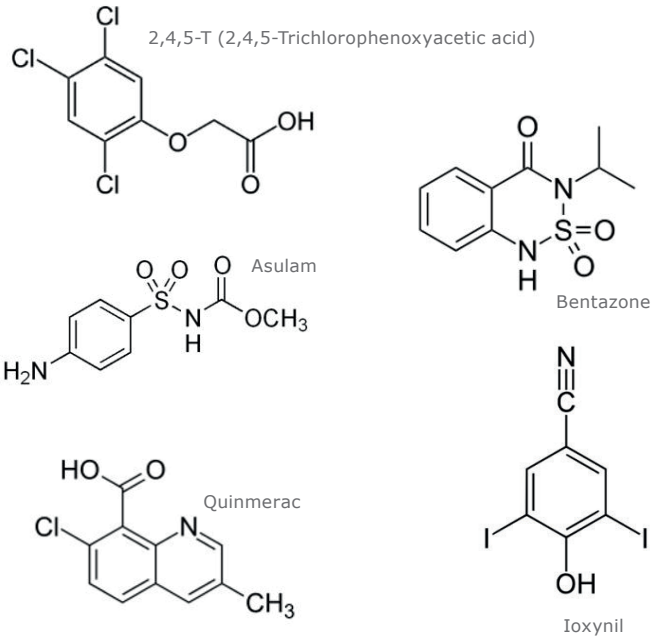

Figure 1. Molecular structures of selected analytes.

Introduction

Acidic herbicides cover a broad range of compounds which are widely used in crop protection and general weed control. They tend to be extremely water soluble, and can easily enter surface and ground waters through natural drainage. Acid herbicides are toxic to humans and aquatic organisms, and are therefore monitored to low ng/L levels in potable and ground waters.

The on-line approach to sample preparation has grown in popularity because of its advantages in improved workflow and reduced sample handling:

- Little or no sample pre-treatment
- Totally automated procedures
- High precision and accuracy
- Elimination of blow down and reconstitution steps
- Reduced solvent use and disposal costs

Trace analysis of organics in water traditionally involves large sample volumes, labor intensive procedures and relatively high use of solvents. The on-line SPE approach uses a simple, well established hardware setup, and fully integrates sample preparation into the analytical workflow. Typical sample volumes of 1-10 mL, and lower solvent usage mean sample collection, transport and handling, along with solvent disposal costs, are much reduced.

This application note describes the use of ISOLUTE® ENV+ on-line cartridges in a fully automated on-line SPE-LC-MS/MS method for extraction and quantification of 16 acid herbicides in drinking and surface water.

Analytes

2,4,5-T, 2,4-D, 2,4-DB, Asulam, Benazolin, Bentazone, Bromoxynil, Dicamba, Dichlorprop, Ioxynil, MCPA, MCPB, Mecoprop, Pentachlorophenol, Quinmerac, Triclopyr

Sample Preparation Procedure

Format:

ISOLUTE® ENV+ On-line SPE cartridge 30 mm x 2.1 mm, part number OSPE-916-32150

Overview

100 µL of pre-treated sample (hard, medium or soft drinking water or surface water) was injected and loaded (trapped) onto the ISOLUTE ENV+ on-line SPE column using a mobile phase consisting of 2% acetonitrile/98% 0.01% formic acid (aq). After 1 min, valve positions were switched to enable transfer of the analytes (in backflush mode) onto the analytical column. Analytes were separated using the gradient conditions shown in Table 1 and analysed using the MS/MS conditions described in Table 3. The total cycle time was 18.5 mins.

Sample Pre-treatment

A 5 mL aliquot of each sample was taken to which internal standard solution and 5% (aq) formic acid (75 µL was added. The samples were vortexed to ensure thorough mixing before 2 mL was transferred to a 2 mL auto sampler vial for analysis

Instrument Set up

SPE-HPLC system: Agilent 1200 series HPLC with 2 binary pumps, switching value, column oven and temperature controlled autosampler (set to 10 ºC).

HPLC column: Zorbax Eclipse plus C18 2.1 x 100mm, 1.8 micron analytical column.

Binary Pump 1

Mobile Phase Channel A: 0.01% Formic acid in high purity water

Mobile Phase Channel B: Acetonitrile

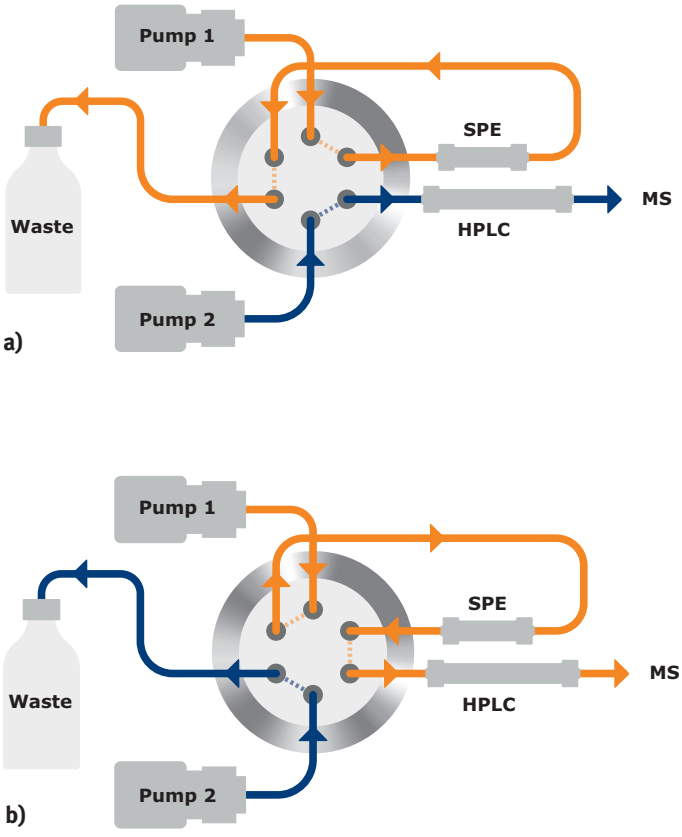

Figure 2. Switching Valve Configuration a) (Top) Valve position 1→2; b) (Bottom) Valve position1→6

HPLC Conditions

Table 1. Pump 1.

Time (Minutes)	%A	%B	Flow Rate	Max. Pressure Limit (bar)
0.00	98	2	0.3 mL/min	600
0.05	98	2	2.0 mL/min	600
0.90	98	2	1.0 mL/min	600
0.95	60	40	0.3 mL/min	600
8.00	60	40	0.3 mL/min	600
12.00	20	80	0.3 mL/min	600
15.00	5	95	0.3 mL/min	600
16.00	5	95	0.3 mL/min	600
16.50	60	40	0.3 mL/min	600

Stop Time: 16.50 Minutes **Post Run:** 2.00

Binary Pump 2

2% Acetonitrile: 98% 0.01% Formic acid in high purity water (isocratic). Flow 0.3 mL/min.

Auto Sampler Injection Parameters

Injection Volume:	100 µL
Injection with needle wash:	Needle wash flush port 5.0 Sec.
Draw Speed:	150 µL/min
Eject Speed:	1000 µL/min
Draw Position:	0.0 mm
Equilibration Time:	3 secs
Sample Flush-Out Factor:	15 times injection volume
Vial/Well Bottom Sensing:	Enabled
Stop time:	As pump

Column Compartment

Temperature:	Thermostatically Controlled at 50 °C
Enable analysis:	when temp. is within ± 0.8 °C (Left & Right)

Table 2. Switching Valve Timing.

Time (min)	Valve Position
0.00	1→2
1.00	1→6
16.5	1→2

Stop Time: As pump

Mass Spectrometer Conditions

Instrument Set up

Agilent 6490 QQQ with ifunnel

Source Parameterss

Sheath Gas Temperature:	300 °C
Sheath Gas Flow:	12 L/min
Drying Gas Temperature:	50 °C
Drying Gas Flow:	14 L/min
Acquisition Mode:	MRM with polarity switching

Table 3. MS/MS Conditions.

	ISTD	Precursor Ion	MS1 Res	Product Ion	MS2 Res	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Asulam		231.1	Unit	155.9	Unit	100	380	10	5	Positive
Quinmerac		222	Wide	204	Unit	200	380	6	5	Positive
Dicamba		218.8	Unit	174.8	Enhanced	500	380	3	2	Negative
Benazolin		244	Unit	170	Unit	200	380	20	4	Positive
Bentazone D_6	X	245	Unit	132.1	Unit	50	380	30	1	Negative
Bentazone		239	Unit	132	Unit	150	380	25	4	Negative
Bromoxynil		275.9	Wide	79	Unit	100	380	40	2	Negative
Ioxynil		369.8	Unit	126.9	Unit	80	380	40	1	Negative
2,4-D $^{13}C_6$	X	224.9	Unit	166.8	Unit	100	380	10	2	Negative
2,4-D		219.1	Unit	161	Unit	100	380	10	1	Negative
MCPA D_6	X	204.9	Unit	147	Unit	100	380	10	4	Negative
MCPA		199	Unit	141	Unit	100	380	15	1	Negative
Triclopyr		254.1	Unit	196.1	Unit	150	380	10	1	Negative
2,4,5-T		253.1	Unit	195.1	Unit	150	380	10	1	Negative
Dichlorprop		232.9	Unit	160.9	Unit	80	380	10	1	Negative
Mecoprop D_3	X	216.1	Unit	143.9	Unit	80	380	15	3	Negative
Mecoprop		213.2	Wide	141.1	Unit	80	380	20	2	Negative
2,4-DB		247.1	Wide	161	Unit	250	380	5	1	Negative
MCPB		226.8	Wide	140.8	Unit	250	380	4	7	Negative
Pentachlorophenol $^{13}C_6$	X	270.9	Unit	35	Unit	200	380	30	5	Negative
Pentachlorophenol		265	Wide	35	Unit	200	380	35	0	Negative

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Table 4. Internal Standard Allocation.

Compound Name	Internal Standard used for Quantitation
2,4,5 T	2,4 D $^{13}C_6$
2,4 D	2,4 D $^{13}C_6$
2,4 DB	2,4 D $^{13}C_6$
Asulam	2,4 D $^{13}C_6$
Benazolin	2,4 D $^{13}C_6$
Bentazone	Bentazone D_6
Bromoxynil	2,4 D $^{13}C_6$
Dicamba	2,4 D $^{13}C_6$
Dichlorprop	Mecoprop D_3
Ioxynil	2,4 D $^{13}C_6$
MCPA	MCPA D_6
MCPB	MCPA D_6
Mecoprop	Mecoprop D_3
Pentachlorophenol	Pentachlorophenol $^{13}C_6$
Quinmerac	2,4 D $^{13}C_6$
Triclopyr	2,4 D $^{13}C_6$

Calibration Curves

Representative samples were spiked at a range of concentrations (shown in table 5) and calibration curves were constructed. Selected examples are shown below. Calibration curves showed a quadratic fit with a r^2 >0.999 achieved for all compounds. See figures 3a–3c for calibration curves for selected analytes.

Table 5. Calibration Range.

Calibration Standards	Concentration (ng/L)
Cal 1	0
Cal 2	10
Cal 3	25
Cal 4	50
Cal 5	75
Cal 6	100
Cal 7	150

Example Calibration Curves

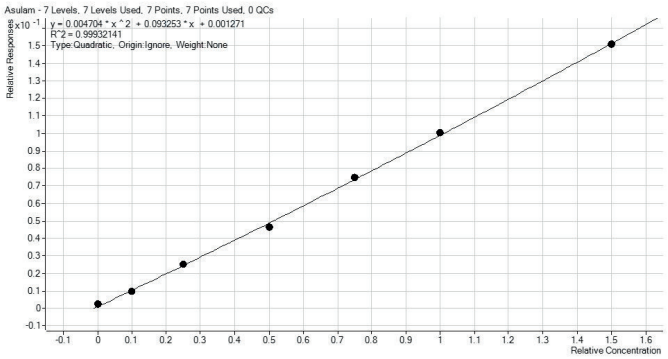

Figure 3a. Asulam.

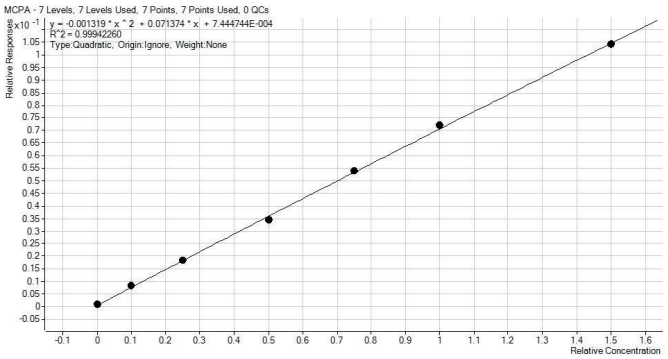

Figure 3b. MCPA.

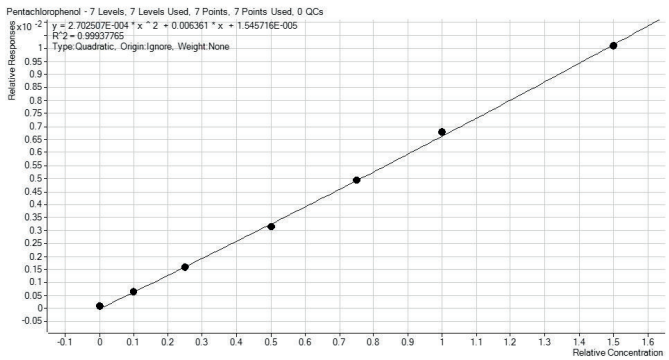

Figure 3c. Pentachlorophenol.

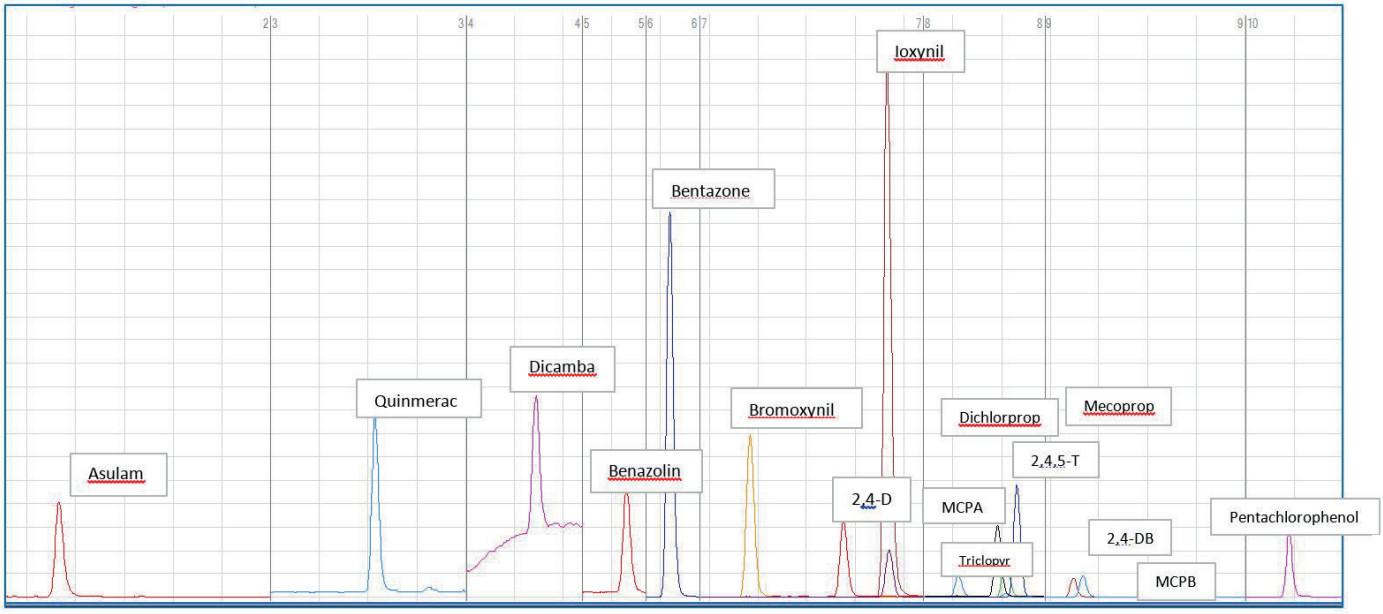

Figure 4. Typical Chromatogram.

Average analyte recovery (n=10), along with measured trueness and precision data for 4 different matrices are shown in tables 6a and 6b.

Precision was calculated by using ((2 x standard deviation)/ Mean) * 100.

Trueness was calculated by using ((Mean – Expected value) / Expected value).

Limits of detection, calculated using 3 times the relative within batch standard deviation of repeat analysis of a low level standard, were in the low ng/l range for all analytes.

Table 6a. Analytical Data (Soft and Medium Water).

	Soft Water				Medium Water			
Analyte	**% Recovery**	**% Trueness**	**% Precision**	**Limit of Detection (ng/L)**	**% Recovery**	**% Trueness**	**% Precision**	**Limit of Detection (ng/L)**
2,4,5-T	103.8	3.77	12.77	0.84	102.9	2.88	17.72	1.11
2,4-D	101.3	1.31	6.88	1.45	99.8	-0.24	12.59	1.74
2,4-DB	101.6	1.64	14.51	1.53	101.0	0.96	14.98	1.48
Asulam	96.3	-3.70	10.19	0.52	101.0	0.97	9.12	2.40
Benazolin	96.1	-3.88	17.19	1.00	96.7	-3.30	18.37	1.10
Bentazone	100.8	0.84	2.84	0.93	99.6	-0.36	4.56	0.80
Bromoxynil	98.0	-2.00	8.84	1.65	95.9	-4.13	11.82	1.52
Dicamba	105.8	5.83	13.81	2.73	104.4	4.36	12.62	3.33
Dichlorprop	98.5	-1.53	16.95	2.12	100.1	0.06	9.74	2.14
Ioxynil	105.4	5.39	18.94	1.33	103.8	3.82	13.64	1.26
MCPA	103.7	3.68	7.02	1.99	104.6	4.55	7.55	2.26
MCPB	102.8	2.80	14.52	1.72	101.1	1.11	16.84	1.38
Mecoprop	98.7	-1.30	6.20	1.71	98.9	-1.12	11.97	0.95
Pentachlorophenol	103.8	3.82	4.25	1.14	104.6	4.55	5.47	1.02
Qunimerac	104.5	4.53	11.57	1.44	101.9	1.92	13.57	1.85
Triclopyr	103.0	3.00	12.36	1.21	100.4	0.42	14.26	3.14

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Table 6b. Analytical Data (Hard and Surface Water).

Analyte	Hard Water				Surface Water			
	% Recovery	% Trueness	% Precision	Limit of Detection (ng/L)	% Recovery	% Trueness	% Precision	Limit of Detection (ng/L)
2,4,5-T	101.2	1.24	9.69	1.75	102.9	2.92	11.53	1.88
2,4-D	99.8	-0.16	8.80	1.83	100.5	0.51	9.72	2.30
2,4-DB	102.4	2.35	15.18	1.25	102.1	2.14	18.22	1.52
Asulam	105.3	5.33	14.33	1.01	92.7	-7.34	7.82	3.35
Benazolin	96.8	-3.19	14.33	2.59	96.3	-3.75	17.32	1.73
Bentazone	99.9	-0.08	6.88	1.30	100.7	0.69	2.25	0.57
Bromoxynil	93.5	-6.55	22.20	0.79	99.5	-0.50	11.27	1.20
Dicamba	103.5	3.54	14.37	1.54	102.2	2.25	8.22	2.85
Dichlorprop	98.9	-1.11	7.69	0.83	100.1	0.14	6.35	1.80
Ioxynil	101.0	0.95	18.03	2.04	102.9	2.95	20.05	1.07
MCPA	101.9	1.88	11.06	1.12	102.0	1.96	5.43	1.28
MCPB	101.5	1.51	15.55	1.07	101.8	1.78	16.78	1.34
Mecoprop	98.6	-1.43	7.09	1.21	98.4	-1.56	6.27	0.69
Pentachlorophenol	103.4	3.37	5.82	1.00	102.3	2.30	4.76	0.97
Qunimerac	99.1	-0.92	13.37	1.81	106.1	6.13	15.02	1.13
Triclopyr	101.9	1.89	9.61	1.50	101.5	1.47	12.23	2.36

Conclusions

- The ISOLUTE® ENV+ On-line SPE cartridge provided reproducible pre-concentration of a wide range of acid herbicides in a variety of water matrices
- Limits of detection in the low ng/mL range are achievable for all analytes
- Reproducibility between batches was seen on the data produced over three separate days. The data was shown to be comparable by using a paired T-test
- No carryover was seen in a randomized batch containing blank and spiked samples.

Ordering Information

Part Number	Description	Quantity
OSPE-916-32150	ISOLUTE® ENV+ On-line SPE Cartridge 30 x 2.1 mm	1

Acknowledgements:

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