Trace Analysis of Chlorinated Herbicides in Water with Online Enrichment

A simple and rapid method with automated sample cleanup

Application Note

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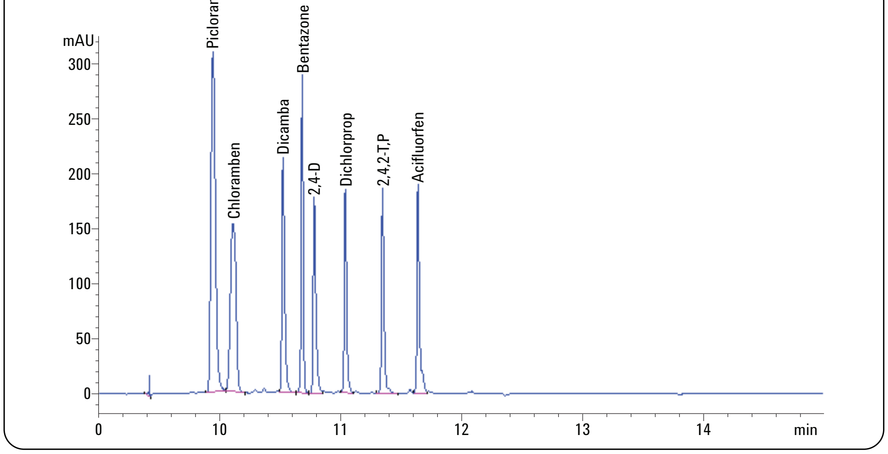
Environmental Analysis



Abstract

This Application Note demonstrates the analysis of eight chlorinated herbicides: picloram, chloramben, dicamba, bentazone, 2,4-D, dichlorprop, 2,4,5,-T,P and aciflourfen in acidified water by online enrichment. The selected herbicides can be analyzed in less than 2 mL of water within a run time of 15 min. This is achieved with an Agilent ZORBAX SB-Aq column for trapping the analytes, an Agilent ZORBAX SB-C18 column for analysis, and a two-position/six-port valve for column switching. The developed method was applied to prepare a standard curve each for picloram and dichlorporp to show the suitability of the proposed method for the quantification of the selected herbicides in water. Automated sample cleanup eliminates laborious solid phase extraction (SPE) protocols for sample preparation. The sensitivity of the Agilent 1260 Infinity LC system can achieve the detection limits prescribed by the US Environmental Protection Agency (EPA) with less than one tenth of the original sample volume specified by the official method.

Introduction

Herbicide contamination in water bodies is monitored to mitigate adverse health impacts. Sensitive methods for detecting and quantifying trace levels of these chemicals are necessary to meet regulatory requirements. Although offline SPE can help enrich target analytes from complex matrices, the methods use expensive cartridges and lengthy protocols making them less suitable for routine use.

This Application Note describes a simple, sensitive, and rugged LC method for the routine analysis of water for quantifying eight chlorinated herbicides. The analytes are initially trapped on an enrichment column fitted on a six port/two-position switching valve. Subsequently, they are eluted from this column and transferred to the analytical column for separation followed by UV detection with the sensitive diode array detector (DAD) of the Agilent 1260 Infinity LC system.

Experimental Conditions

System

The Agilent LC system consisted of the following modules,

- Agilent 1260 Infinity Binary Pump (G1312B)
- Agilent 1260 Infinity Micro Degasser (G1379B)
- Agilent 1260 Infinity Autosampler (G1329B) modified with a Multidraw kit (G1313-68711) and a 900 µL injection Upgrade kit (G1363A)
- Agilent 1260 Infinity Thermostat (G1330B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)
- Two-Position/ Six-Port column switching valve (5067-4646)
- Agilent 1260 Infinity Diode Array Detector (G4212B), with Max-Light 10 mm High Sensitivity flow cell
- Software: ChemStation B.04.02

Chromatographic conditions

Columns	Enrichment/Cleaning: Agilent ZORBAX RRHT SB-Aq 2.1 X 30 mm, 1.8 µm (p/n 824700-914) Analytical: Agilent ZORBAX RRHD SB-C18 2.1 x 50 mm, 1.8 µm (p/n 857700-902)
Mobile phase	A = Phosphoric acid (25 mM, in Milli-Q water) B = Acetonitrile
Injection volume	1800 µL

Enrichment/Loading conditions

Flow rate	0.5 mL/min	
Gradient	Time(min)	% Acetonitrile
	0	0
	8.1	10
	9.0	10
	9.1	0
	Stop time: no limit	

Elution and analysis conditions

Flow rate	0.61 mL/min	
Gradient	Time (min)	% Acetonitrile
	0.0	20
	9.0	20
	12.5	80
	13.5	80
	14.0	20
	Stop time: 15 min	
Column temperature	40 °C on both sides	
Column switching	0 min: Load/Equilibrate (valve position 1) 9 min: Elute/Equilibrate (valve position 2) 14 min: Load/Equilibrate (valve position 1)	
Detection	230 nm, 8 nm BW; Ref: No; PW > 0.062 s (80 Hz); Slit Width: 8 nm	

Samples

Picloram; chloramben; dicamba; bentazone; 2, 4-D; dichlorprop; 2,4,5-T,P and acifluorfen were purchased from Sigma Aldrich. Stock solutions were prepared in methanol and samples diluted in 25 mM phosphoric acid. Solutions containing 0.5, 1, 1.5, 3, 4.5, 9, 90, 180 and 240 ppb of picloram and dichlorprop were used to prepared the calibraiton curves. A solution of 50 ppb of each of the selected herbicides was used to examine the method reproducibility.

Results

The US EPA Method 555[1] describes the use of online sample enrichment for the analysis of chlorinated herbicides in 20 mL of water. An earlier Application Note[2] describes in detail the advantages of using sub-2-µm columns for rapid and sensitive analysis of smaller sample volumes. This Application Note describes a method with automated sample cleanup for the assay of eight chlorinated herbicides using less than 2 mL of water.

An Agilent 1260 Infinity Binary Pump was used to load 1800 µL of the sample on to the enrichment (or cleaning) column with 100% of mobile phase A. Since the maximum volume of sample that can be injected per draw using the Agilent 1260 Infinity Autosampler is 100 µL, it was modified with a Multidraw kit and a 900-µL Injection Upgrade kit to allow rapid large volume injections. With this hardware modification, 900 µL of the sample were drawn twice, filled in the sample loop and subsequently injected on the ZORBAX SB–Aq column, which can withstand large aqueous loads. After a load time of 8 min, the column was washed with 10% acetonitrile to prevent the buildup of organic impurities. During this step the analytes were trapped on the loading column while polar matrix constituents were washed away leading to sample cleaning.

A two-position/six-port valve was

installed on the thermostatted column compartment. After the loading cycle, the valve was switched and the flow from the second 1260 Infinity Binary Pump was pumped through the enrichment column. The trapped analytes were eluted and carried to the ZORBAX SB-C18 analytical column for separation prior to detection using the 1260 Infinity Diode Array Detector equipped with the proprietary Agilent Max-Light flow cell.

Valve position and pump flows

Figure 1 shows the flow path throughout the two valve positions. During sample loading (valve position A) the flow from one of the 1260 Infinity Binary Pumps (Pump A) flows through the enrichment column while the flow from the second 1260 Infinity Binary Pump (Pump B) flows through the analytical column under initial conditions, maintaining equilibrium. During the elute/analyze cycle (valve position B), the flow from the first 1260 Infinity Binary Pump (Pump A) is diverted to waste while the flow from the second 1260 Binary Pump (Pump B) passes through the enrichment column to elute the trapped analytes to the analytical column for analysis.

Sample volume is decreased by a factor of 20 (Table 1) by reducing the column dimensions from 4.6 × 250 mm (the EPA method) to 2.1 x 50 mm (present method). Although 900 µL of the sample can theoretically be used, in the present study 1800 µL were used to improve the detection limits of the assay. This is one order of magnitude lower than 20 mL, the volume of the sample prescribed by the US EPA Method 555. In addition, the smaller particle size of the packing material in the 2.1 × 50 mm column also helps to improve the sensitivity of the method.

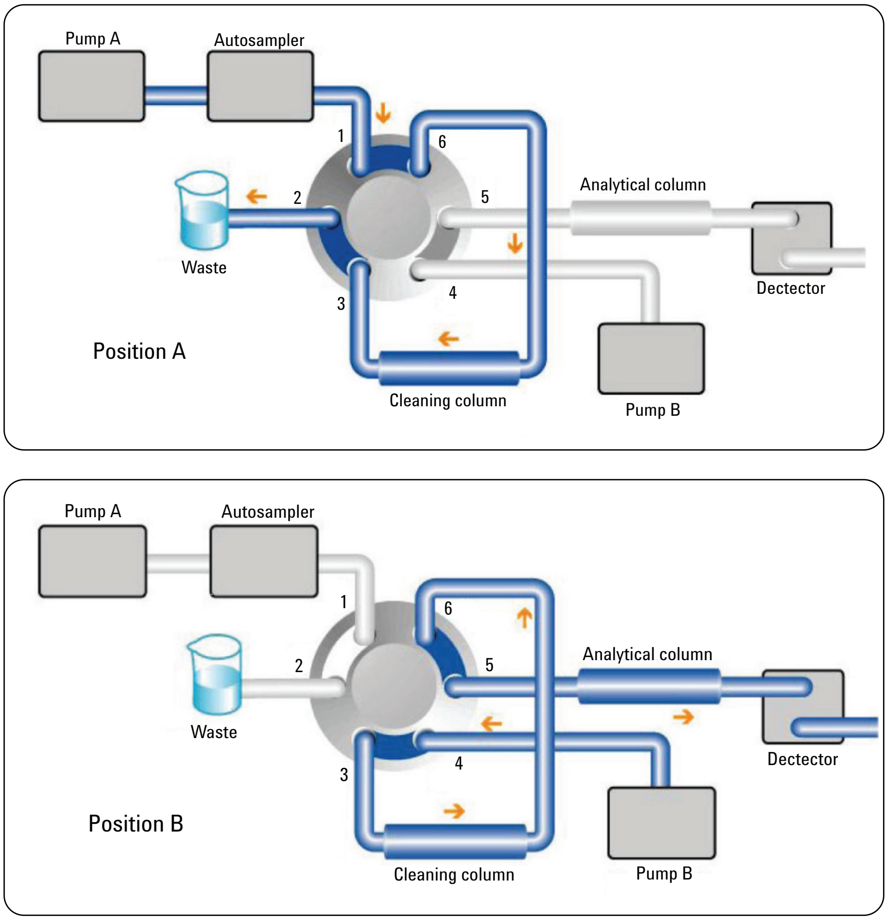


Figure 1
Valve positions during online enrichment.

Column dimensions	Column bed volume	Trace enrichment volume	Sample reduction factor
4.6 x 250 mm	2.50 mL	20 mL	na
3.0 x 150 mm	0.63 mL	5 mL	4
2.1 x 100 mm	0.21 mL	1.7 mL	11.7
2.1 x 50 mm	0.10 mL	0.9 mL	20

Table 1
Column dimensions and corresponding injection volumes.

At the end of the loading cycle, when the flow path is switched, the elute/analysis cycle begins. This process completes in less than 3 minutes from the start of the second cycle (Figure 2)

The RSD values for the retention times, areas and peak heights of all the analytes obtained from three replicate injections of the sample containing 50 ppb of each analyte were all less than 1%. Excellent reproducibility makes this method suitable for the routine analysis of water samples in environmental laboratories.

The applicability of the method for quantification of the selected herbicides in water was tested by developing standard curves for two of the analytes: picloram and dichlorprop. Figures 3a, 3b and 4a, 4b illustrate that the area responses correlate well with the concentrations for both compounds. The calibration curves prepared on three independent days were found to be linear on all occasions with correlation coefficient values being ≥ 0.99.

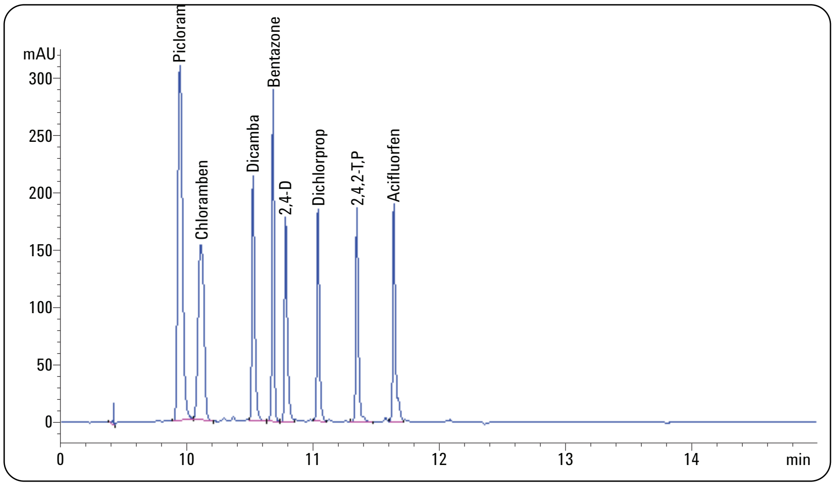


Figure 2
Chromatogram of a sample containing 50 ppb of each of the eight analytes.

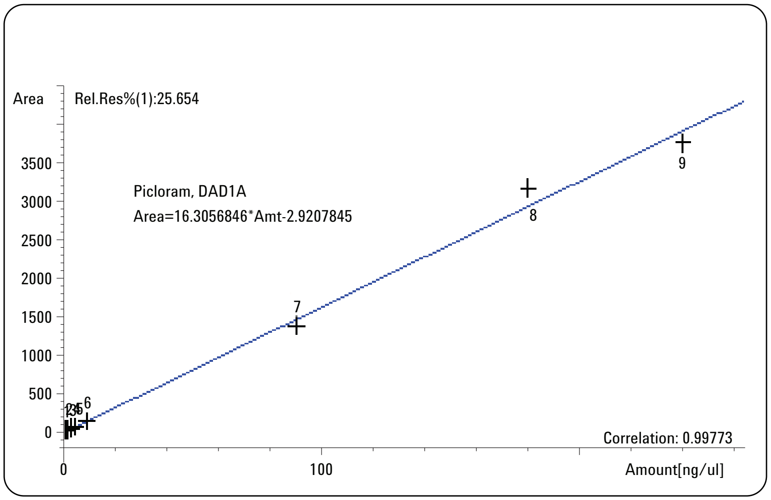


Figure 3a
Calibration curve for picloram.

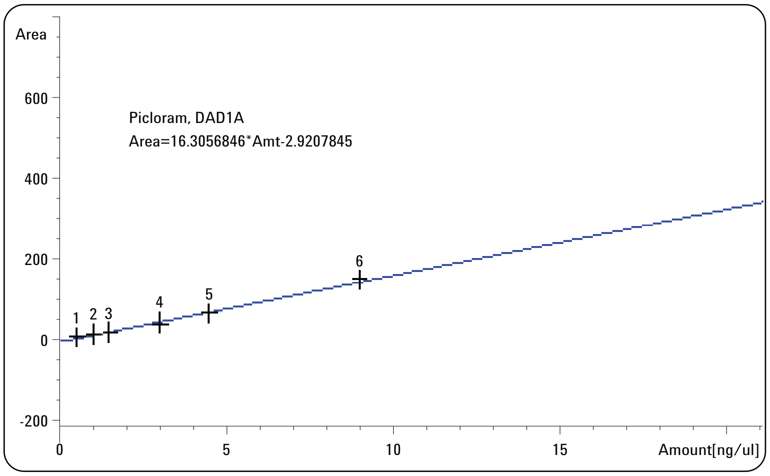


Figure 3b
Zoomed in view of the lower end of the calibration curve.

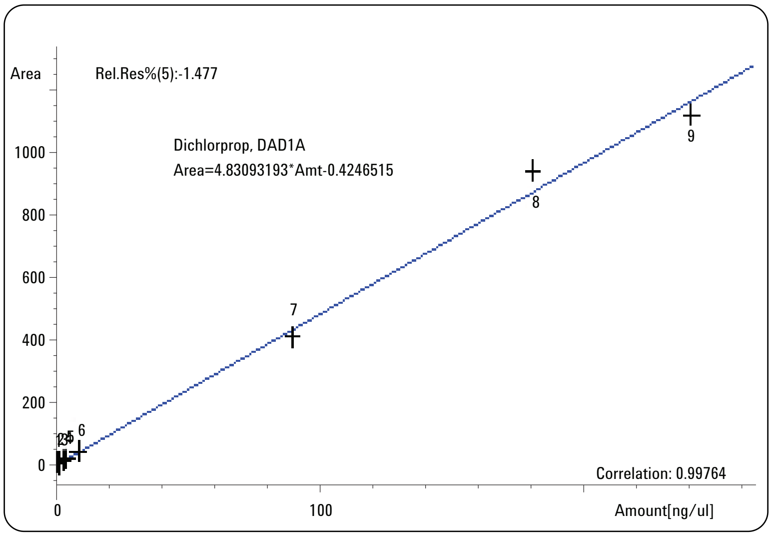


Figure 4a
Calibration curve for dichlorprop.

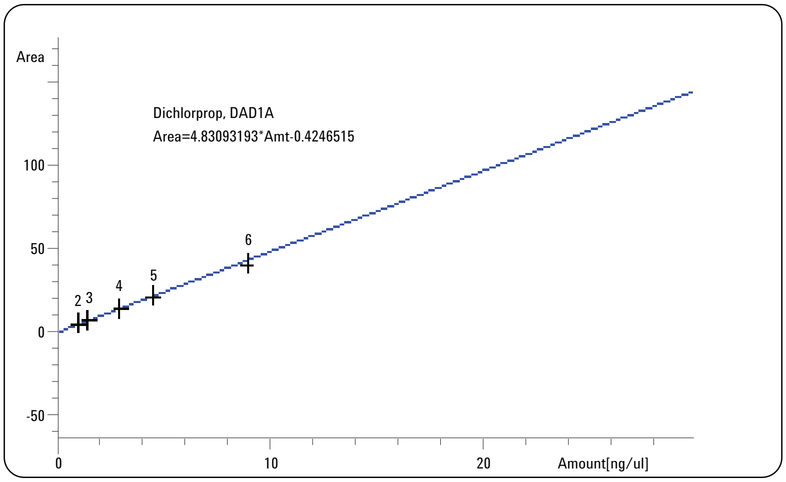


Figure 4b
Zoomed in view of the lower end of the calibration curve.

Table 2 shows a set of peak areas at the various concentrations used to construct the calibration curves for the two compounds.

Concentration (ng/mL)	Area (Picloram)	Area (Dichlorprop)
0.5	6.57	-
1.0	13.70	4.91
1.5	19.28	6.83
3.0	40.58	13.65
4.5	65.40	21.00
9	148.34	40.35
90	1375.7	410.11
180	3168.9	939.31
240	3766.2	1115.60

Table 2
Area responses versus concentrations used for constructing the calibration curves.

The concentration value at which the S/N ratio was ≥ 10 was assigned as the lowest level on the calibration curve for the compound being studied. In the present study, the S/N ratio was calculated by dividing the peak height by the peak-to-peak noise between 14.5 and 15 min. For picloram, the average S/N from three replicate analyses at 0.5 ppb level was calculated to be 24, while for dichlorprop the average S/N for three replicate analyses at 1 ppb level was found to be 30. Therefore, these concentrations match the US EPA Method 555 recommended limits (0.5 ng/mL for picloram and 1.7 ng/mL for dichlorprop) and were assigned as the limits of quantitation by this method. The baseline subtracted chromatograms at the LOD level for picloram is shown in Figure 5.

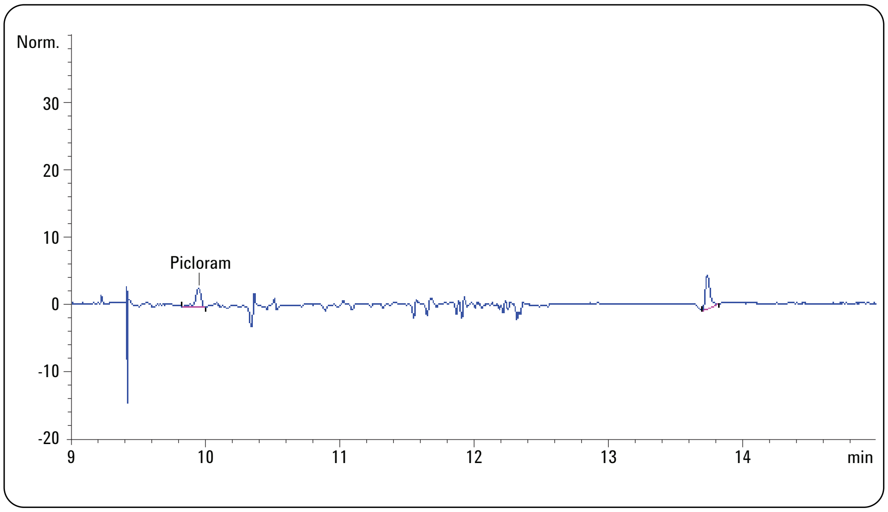


Figure 5
Baseline subtracted chromatograms at 0.5 ng/mL concentration level (LOD of picloram).

Conclusion

This Application Note shows that the Agilent 1260 Infinity LC system can detect and quantify chlorinated herbicides in acidified water. The instrumentation and columns are capable of withstanding higher backpressures of up to 600 bar, which allows the quantification of the analytes within a run time of 15 min. This new method can achieve the detection limits prescribed by the US EPA even with the smaller sample volume of 1.8 mL due to the highly efficient sub-2-µm trace enrichment and analysis columns, and the higher sensitivity of the Agilent 1260 Infinity Diode Array Detector equipped with Max-Light Flow Cell. The good reproducibility observed with the method developed makes the assay suitable for routine use by environmental laboratories.

References

1. James W. Eichelberger and Winslow J. Bashe, US EPA Method 555, "Determination of chlorinated acids in water by High performance Liquid chromatography with a photodiode array ultraviolet detector", Revision 1, August 1992, J Environmental Monitoring Systems Laboratory, Office of Research and Development , U. S. Environmental Protection Agency, Cincinnati, Ohio 45268.
2. Rapid analysis of herbicides by rapid resolution LC with online trace enrichment, Agilent Technologies, publication 5989-5176, March 30, 2007.

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