

UHPLC Separation of Triazine Herbicides at Elevated Temperature

Dave Thomas, Thermo Fisher Scientific, San Jose, CA USA

Key Words

- Accela UHPLC
- Environmental Analysis
- Herbicides
- High Temperature
- Hypercarb LC Column
- Triazines

Goal:

Increase throughput of the HPLC method for triazine herbicides by employing ultra high-speed liquid chromatography at elevated temperature on a heat stable Hypercarb column.

Introduction

Temperature is a key variable in high performance liquid chromatography (HPLC), influencing solute diffusion rates, mobile phase viscosity, and solubility. For example, as column temperature increases, analyte diffusion increases. Increased analyte diffusion generally leads to an increase in the optimum linear velocity of the separation, so that equivalent chromatographic efficiency and resolution can be achieved at a higher flow rate. Furthermore, elevating the temperature reduces the operating backpressure. The net result is that separations can be performed faster without exceeding the pressure limitations of the instrument.

This application uses a porous graphitic carbon stationary phase thermostatted in a high temperature column oven to separate triazine herbicides 5 to 10 times faster than is typical with conventional HPLC. The triazines and degradation products are separated on the Accela High Speed Liquid Chromatograph in 2 minutes on a Hypercarb™ 3 µm, 1 x 100 mm column operated at 160 °C. This application note also documents the performance of the high temperature liquid chromatographic method, including precision of retention time and peak area, resolution, and spike recovery from several environmental water matrices.

Experimental

Instrumentation

Thermo Scientific Accela HPLC system with PDA Detector
Thermo Scientific ChromQuest 5.0 Chromatography Data System (CDS)
Polaratherm Series 9000 Total Temperature Controller (Selerity Technologies)

Chromatographic conditions

Column:	Thermo Scientific Hypercarb 3.0 µm, 1 x 100 mm (35003-101046)		
Mobile phase:	A: water	B: acetonitrile	
Gradient:	Time	%A	%B
	0.00	75	25
	1.00	70	30
	2.20	10	90
	2.30	75	25
	4.00	75	25
Flow rate:	500 µL/min		
Detector:	PDA, 238 nm, 10-mm flow cell, 11nm bw, 20 Hz, 0s rise time		
Column temp.:	160 °C (housed in Selerity temperature controller)		
Injection:	5 µL sample loop, 2 µL partial loop injection		
	Syringe Speed: 4 µL/sec		
	Flush Speed: 100 µL/sec		
	Flush Volume: 400 µL		
	Wash Volume: 200 µL		
	Flush/Wash source: Bottle with 90:10 methanol:water		

Chemicals

Water, LC/MS-grade	Fisher Scientific W6
Acetonitrile, LC/MS-grade	Fisher Scientific A998
Methanol, LC/MS-grade	Fisher Scientific A456
Atrazine	Supelco 49085
Ametryn	ULTRA PST-024
Cyanazine	ULTRA PST-1360
Deisopropylatrazine, 1000 mg/L	SPEX CertiPrep S-1135
Desethylatrazine, 1000 mg/L	SPEX CertiPrep S-1145
Propanil, 1000 mg/L	SPEX CertiPrep S-3155
Propazine	ULTRA PST-850
Prometryn	ULTRA PST-840
Simazine	ULTRA PST-1130
Simetryn	Chem Service PS-381

Consumables

Autosampler vials, 1.8 mL glass, yellow septa	Thermo Scientific A4954-010
Backpressure assembly	Upchurch P-788
Ferrules, high temperature	Selerity Technologies BM0054
Mixer, 50 µL in-line static	Thermo Scientific 109-99-032
Mobile Phase Preheater, 0.005" x 70 cm	Selerity Technologies AD104
Syringe filters, 0.45 µm Nylon	Thermo Scientific A5307-010
Sample Loop, 5 µL	Thermo Scientific 109-99-007

Mobile Phase

Proportioned mobile phase: Filled Solvent Reservoir Bottle A of the Accela pump with fresh HPLC-grade water and purged the solvent line with at least 30 mL of the water. Connected a fresh bottle of HPLC-grade acetonitrile to Reservoir B and purged as above.

Calibration Standards

Individual Stock Solutions, 1000 mg/L: Accurately weighed 10 mg (0.010 g) of each neat compound into a 10-mL volumetric flask, added 5 mL acetonitrile, and sonicated to dissolve. Brought to volume with acetonitrile and mix. Used desethyl atrazine, deisopropyl atrazine and propanil, purchased as solutions of 1000 mg/L in methanol, as received.

Combined Intermediate Standard 100 mg/L: Used a calibrated pipette to deliver 1000 μ L of each individual stock solution to a 10-mL volumetric flask. Brought to volume with acetonitrile and mix.

Calibration standards: Used a calibrated pipette to dilute the intermediate standard with mobile phase in volumetric glassware to 30, 10, 3, 1, 0.3, 0.1, and 0.03 mg/L.

Samples

Samples of surface water (Salinas River, Monterey County, CA), ground water (domestic well, Santa Cruz county, CA), and drinking water (San Jose, CA tap water) were collected in accordance with established procedures, stored at 4 - 8 °C, and were filtered through a 0.45 μ m nylon syringe filter into a glass autosampler vial before analysis.

System Preparation

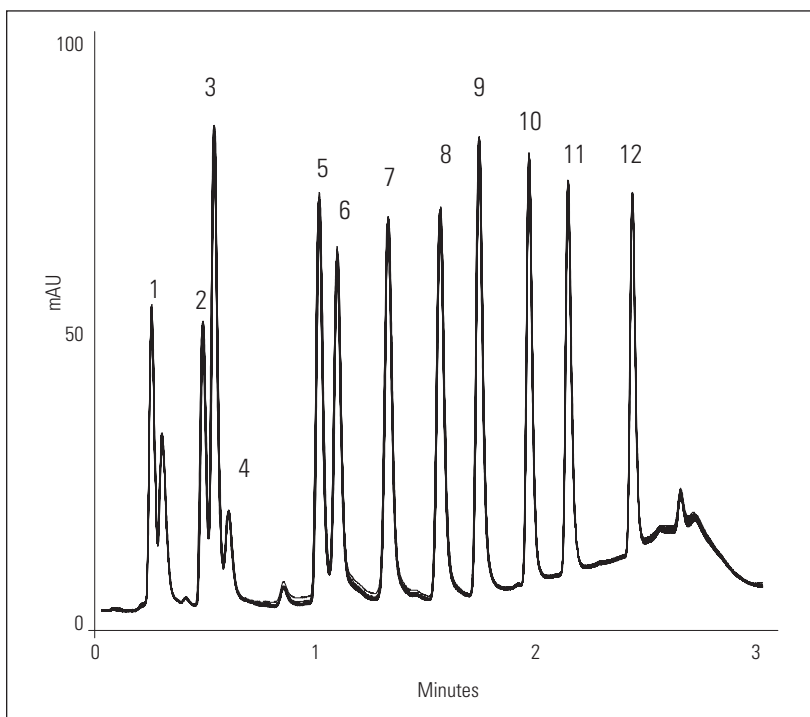
To ensure good performance of this application, prepare the system as directed in Appendix A.

Results

Separation of seven triazine herbicides, two triazine degradation products often found in environmental samples, and propanil is shown in Fig 1. To optimize this separation, we adjusted the mobile phase composition to elute the first analyte with a capacity factor $k' > 2$, thereby improving resolution of the target analytes from sample matrix junk. Analytes spanning a wide range of polarity are well resolved by the combination of high temperature, solvent gradient, and the selectivity of the Hypercarb stationary phase. Note that because of the reduced viscosity of the mobile phase at 160 °C, this separation occurs at a linear flow rate of 15 mm/s — equivalent to a flow rate of over 10 mL/min on a 4.6 mm i.d. column. The system backpressure under these conditions is less than 4000 psi (272 bar).

Method performance is characterized by peak resolution, linear calibration range, limits of detection, and precision of retention time and peak area, as summarized in Table 2. MDLs for each analyte were determined by performing seven replicate injections of LC/MS-grade water fortified at a concentration of three to five times the estimated instrument detection limits, calculating the standard deviation of the measured concentration, and using the equation given in the figure caption. Note the good precision of retention time and peak area, as this reflects the temperature stability maintained by the high temperature oven.

We analyzed several environmental water samples to demonstrate the efficacy of this method with real matrices. Samples of surface, ground and municipal drinking water were analyzed before and after fortification with a known amount of each target analyte. Spike recovery was calculated as the amount of each analyte found in the spiked sample divided by the amount expected (i.e., the amount determined in the blank plus the amount added in the spike). Even with the dirtiest matrix, Salinas River water, the target analytes are well separated from the early eluting matrix peaks (Figure 2) and recovery of the spiked analytes exceeds 80% (Table 3).



Column:	Hypercarb 3 μ m, 100 x 1 mm	
Temperature:	160 $^{\circ}$ C	
Flow rate:	500 μ L/min	
Detector:	Accela PDA at 215nm, 20Hz, 0s rise time	
Injection:	2 μ L partial loop from 5 μ L loop	
Solvents:	A: Water	
	B: Acetonitrile	
Gradient:	Time (min)	A% B%
	0.00	75 25
	1.00	70 30
	2.20	15 85
	2.10	75 25
	4.00	75 25
Samples:	triazines and propanil in 20% acetonitrile	
Peaks:	<ol style="list-style-type: none"> 1. Melamine 2. Unknown 3. Deisopropylatrazine 4. Desethylatrazine 5. Cyanazine 6. Propazine 7. Prometryn 8. Atrazine 9. Ametryn 10. Simazine 11. Simetryn 12. Propanil 	

Figure 1: Separation of triazine herbicides, degradation products, and propanil on the Accela High Speed LC by reversed-phase chromatography with UV absorbance detection at 215 nm. Peaks: see Figure. Sample: overlay of 30 injections of triazines in HPLC-grade water with 20% acetonitrile. Conditions: see text for details.

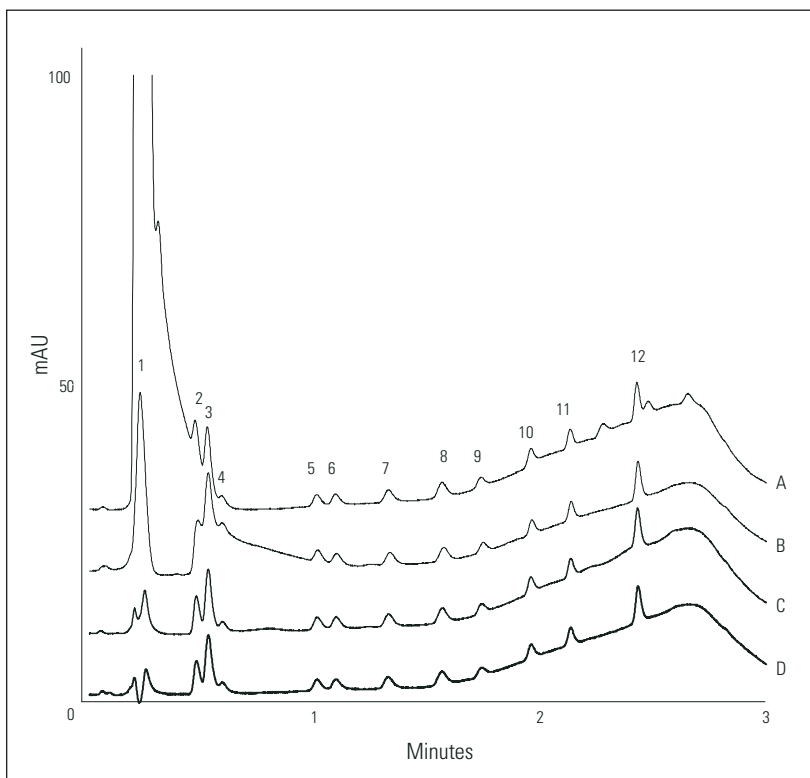


Figure 2: Chromatograms of four environmental water samples spiked with 200 μ g/L each of triazines and propanil. Chromatograms obtained on the Accela High Speed LC by reversed phase chromatography with UV absorbance detection at 215 nm. Peaks: see Figure 1. Samples: top trace A, surface water (Salinas River); trace B, ground water (Simoes' well); trace C, drinking water (San Jose tap); bottom trace D, HPLC-grade water. Conditions: see text for details.

Table 1. Useful properties of some triazine herbicides and degradation products^a

CAS#	4-deethyl atrazine 6190-65-4	6-deisopropyl atrazine 1007-28-9	Cyanazine 21725-46-2	Propazine 139-40-2	Prometryn 7287-19-6
Formula	C ₆ H ₁₀ ClN ₅	C ₅ H ₈ ClN ₅	C ₉ H ₁₃ ClN ₆	C ₉ H ₁₆ ClN ₅	C ₁₀ H ₁₉ N ₅ S
MW (g/mol)	187.633	173.606	240.697	229.713	241.361
pKa			0.87	1.7	4.05
Log P _{o-w}	1.51	1.15	2.22	2.93	3.51
Water solubility, mg/L	3200	670	170	8.6	33
MeOH solubility, g/L			45 (ethanol)	6.2 (toluene)	160

CAS#	Atrazine 1912-24-9	Ametryn 834-12-8	Simazine 122-34-9	Simetryn 1014-70-6	Propanil 709-98-8
Formula	C ₈ H ₁₄ ClN ₅	C ₉ H ₁₇ N ₅ S	C ₇ H ₁₂ ClN ₅	C ₈ H ₁₅ N ₅ S	C ₈ H ₉ Cl ₂ NO
MW (g/mol)	215.687	227.334	201.66	213.307	218.082
pKa	1.7	4.1	1.62	4	2.29
Log P _{o-w}	2.61	2.98	2.18	2.8	3.07
Water solubility, mg/L	34.7	209	6.2	450	152
MeOH solubility, g/L	18	510	400		540

^a<http://toxnet.nlm.nih.gov>

Table 2. Performance of high temperature method for triazines performed on Hypercarb 3 μm, 1 x 100 mm column at 160 °C.

Analyte	k' ^a	R ^a	Linear range, mg/L	r ²	MDL ^b μg/L	Precision, Retention Time % RSD ^c	Precision, Peak Area % RSD ^c
deisopropylatrazine	2.4	1.1	0.03 – 10	0.9999	6	0.25	0.39
desethylatrazine	2.8	1.2	0.03 – 30	0.9999	16	0.22	0.89
cyanazine	5.6	7.2	0.03 – 30	0.9995	40	0.16	0.47
propazine	6.1	1.5	0.03 – 30	0.9996	23	0.13	0.82
atrazine	7.7	3.9	0.03 – 30	0.9995	14	0.10	0.87
simazine	9.2	3.9	0.03 – 30	0.9994	30	0.09	0.92
prometryn	10.4	3.0	0.03 – 30	0.9999	32	0.08	0.97
ametryn	11.9	4.3	0.03 – 100	0.9995	8	0.04	0.64
simetryn	13.1	3.7	0.03 – 100	0.9999	16	0.04	0.57
propanil	15.1	6.3	0.03 – 30	1.0000	25	0.02	0.42

^a Capacity factor (k') and Resolution (R) calculated according to Reference 1.^b Detection limit MDL = $\sigma t_{s,99}$ where $t_{s,99} = 3.14$ for n = 7 replicates of the standard.^c for n = 30 replicates.

Table 3. Percent recovery of analytes spiked into selected environmental water matrices. n = 3 replicates.

Analyte	HPLC water	Drinking water	Ground water	Surface water
deisopropylatrazine	94.0	104	104	93.0
desethylatrazine	93.0	110	98.5	85.0
cyanazine	92.5	102	94.0	97.0
propazine	97.0	106	104	101
atrazine	94.5	104	101	98.5
simazine	98.0	102	100	103
prometryn	104	117	116	110
ametryn	97.0	104	101	98.5
simetryn	99.0	98.5	104	100
propanil	101	103	104	80.0

Conclusion

A separation performed at 160 °C on the Accela high speed chromatography system equipped with a heat stable Hypercarb column and high temperature column oven resolves 11 triazine herbicides in about two minutes with retention time and peak area precision better than 1% RSD for thirty replicates.

References

1. United States Pharmacopeia 30-National Formulary 25, United States Pharmacopeia, Rockville, Maryland 20852-1790, USA.

Suppliers

Chem Service, West Chester, PA, USA (<http://www.chemservice.com>)
 Selerity Technologies, Inc., Salt Lake City, UT, USA
 Sigma-Aldrich, St. Lois, MO, USA (<http://www.sigmaaldrich.com>)
 Supelco, Bellefonte, PA, USA (<http://www.sigmaaldrich.com>)
 Thermo Fisher Scientific, Waltham, MA, USA (<http://www.thermofisher.com>)
 ULTRA Scientific, No. Kingstown, RI, USA (<http://www.ultrasci.com>)

Appendix A.

System Preparation

Pump: Always plumb the Accela system with precut and polished 0.005" i.d. high-pressure tubing and high pressure fittings as shown in Figure 15 of the Accela Pump Hardware Manual (Document 60157-97000 Revision B). For all tubing connections that you make, ensure that the tubing end is square-cut and burr-free. Firmly push the tubing into the injection valve port as you tighten the high-pressure fitting in order to maximize peak efficiency. Prime the pulse dampener and purge the solvent lines as instructed in Chapter 4 of the Accela Pump manual. Verify that the pump is performing well by monitoring the pressure pulsation and by testing the pump proportioning accuracy as described in Chapter 5 of the pump manual. If your Accela pump does not include an inline 35 μ L dynamic mixer, then install a 50 μ L static mixer between the inline high pressure filter and the Accela AS mobile phase preheater.

AS: Open the Instrument Configuration and verify that the Accela AS Configuration entry for "Dead volume" is correct (the calibrated volume in μ L written on the transfer tubing between the injection port and injection valve). Verify that the entry for "Loop size" is correct for the currently installed sample loop. Fill the Flush reservoir with 90:10 (v/v) methanol:water and flush the syringe with solvent to purge any air bubbles from the syringe and

tubing. Use the Wash/Flush conditions specified under "Conditions" to ensure low carryover between injections. Consult the Accela Getting Connected manual (Document 60057-97001 Revision A) for details.

Polaratherm Column Oven: Install the Total Temperature Controller according to the Polaratherm Series 9000 Installation and Operation Manual. Install the Hypercarb, 3 μ m 1 x 100 mm column, by using a 70-cm length of precut and polished 0.005" i.d. high-pressure tubing with mobile phase preheater. It is important to use a heat stable Hypercarb column as this does not contain any PEEK components that will degrade at the temperatures used in this method. Use the high temperature graphite/Vespel ferrules and fittings described in the Series 9000 manual. Ensure that the tubing is fully pushed into the column inlet when you tighten the high-pressure fitting.

Detector: Use a 10 mm light-pipe flow cell. Install a 250 psi backpressure regulator after the flow cell outlet to suppress bubble formation in the flow cell. Verify that the deuterium lamp has been used for less than 2000 hours.

Use Direct Control or a downloaded method to equilibrate the Accela system under the conditions shown above. Create a method based on these operating conditions and then create a sequence to make several injections of HPLC grade water. The system is ready to run standards and samples when the peak-to-peak baseline oscillation is between 50 – 200 μ AU/min (average of 10 1-min segments) and no significant peaks elute in the retention time window of the analytes.

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