Pesticide Analysis using an Agilent 1290 Infinity LC System with an Agilent 6140 Single Quadrupole LC/MS System

Application Note

Environmental

Author

Rob Sample
Littleover
Derby, DE23 3XH, UK

Edgar Naegele
Agilent Technologies
Waldbronn, Germany

Abstract

This Application Note describes the use of the Agilent 1290 Infinity LC system in combination with the Agilent 6140 Single Quadrupole LC/MS system for cost-effective LC/MS analysis of pesticides in clean to moderately dirty water samples such as river water or drinking water. A separation method for the analysis of water samples with relatively clean matrices such as those that might be prepared by a simple concentration and cleanup step using a solid phase extraction (SPE) cartridge was developed. This demonstrates how the run time for such a method can be shortened.

Introduction

In pesticide analysis literature, there are many examples of liquid chromatography combined with triple quadrupole mass spectrometry. While these techniques are extremely sensitive for detecting pesticides in 'dirty' matrices such as soil or crop extracts the single quadrupole MS detector is a more cost-effective method for the analysis of clean to moderately dirty samples such as drinking water or river water. Addition of a single quadrupole MS detector to a UV diode array detector-based system is a common first step into mass spectrometric detection. This Application Note develops a separation method for the analysis of water samples with relatively clean matrices, prepared by a simple concentration and cleanup step using a solid phase extraction (SPE) cartridge. It also demonstrates how the run time for this method can be shortened. Simple techniques for converting methods to faster methods are illustrated.

Agilent Technologies

Experimental

All analyses were performed using the Agilent 1290 Infinity LC system including binary pump, autosampler, thermostatted column compartment and diode array detector in series with the Agilent 6140 Single Quadrupole LC/MS system equipped with an atmospheric pressure electrospray ionization (AP-ESI) source.

Agilent ZORBAX Rapid Resolution High Definition (RRHD), 1.8 µm columns were used with the Agilent 1290 Infinity LC system. The column dimensions were chosen to handle operating pressures up to 1200 bar. For the initial method development, an Agilent ZORBAX StableBond C18 RRHD 100 mm × 2.1 mm, 1.8 µm column was chosen.

Samples

A standard mixture containing 10 ng/µL each of 17 pesticides in acetonitrile solution was obtained from Dr. Ehrenstorfer GmbH (Pesticide Mix 44, part no. 18000044 - Dr. Ehrenstorfer GmbH Bgm.-Schlosser-Str. 6 A, 86199 Augsburg, Germany). Aliquots of this solution were diluted to 10% with water to prepare standards containing 1 ng/µL (1 ppm) of each component for development of the separation method.

The mixture contained herbicides from the triazine, phenylurea and chloroacetanilide classes. The components are listed in Table 1. These components are all expected to form positive ions under the acidic conditions of the mobile phase.

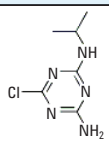
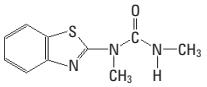
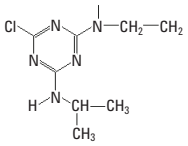
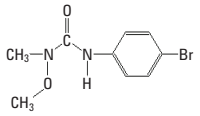
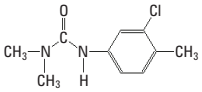
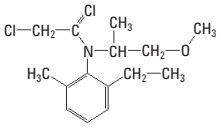
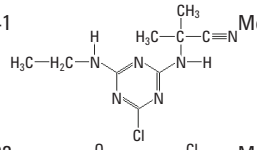
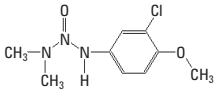
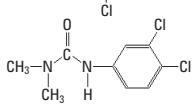
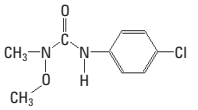
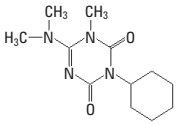
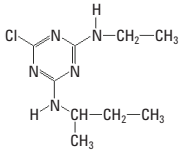
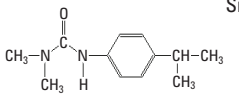
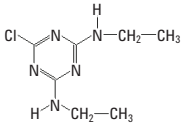
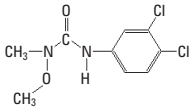
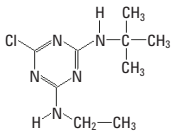
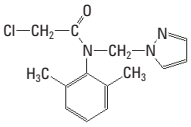
Component	m/z $[M+H]^+$	Structure	Component	m/z $[M+H]^+$	Structure
Atrazine-desethyl	188		Methabenzthiazuron (also known as methibenzuron)	222	
Atrazine	216		Metobromuron	259	
Chlorotoluron	213		Metolachlor	284	
Cyanazine	241		Metoxuron	229	
Diuron	233		Monolinuron	215	
Hexazinone	253		Sebuthylazine	230	
Isoproturon	207		Simazine	202	
Linuron	249		Terbuthylazine	230	
Metazachlor	278				

Table 1
Components in pesticide test mixture.

Results and discussion

Standard method development

An Agilent ZORBAX StableBond C18 RRHD 100 mm × 2.1 mm, 1.8 µm column was chosen for the separation method development. Conditions were adjusted to obtain a target analysis of the 17 components in about 10 minutes. Formic acid (0.1% v/v) was added to the acetonitrile/water mobile phase to promote positive ion formation for mass spectrometric detection using the atmospheric pressure electrospray source.

Fifteen of the seventeen components were separated in 10 minutes with the remaining two (diuron and isoproturon), coeluting at about 5.8 minutes. An advantage of using the mass spectrometric detector is that two overlapping peaks may be separated by their mass/charge (*m/z*) ratios into different chromatograms. The maximum pressure observed during the gradient separation was 546 bar, which is well within the operating range of 1200 bar. Table 2 shows the peak assignments and Table 3 summarizes the LC/MS method conditions used in this Application Note.

The Agilent 6140 Single Quadrupole LC/MS system can output four chromatographic signals simultaneously (MSD1 to MSD4). MSD1 was set to scan mode so that the mass spectrum of each peak in the resulting total ion current (TIC) chromatogram (Figure 1), could be used to verify that molecular ions ($[M+H]^+$) were produced for each component in order to identify and quantify the peaks. High quality spectra were obtained for all components and two examples are shown in Figures 4a and 4b.

For quantification of components, the use of TICs from selected ion monitoring (SIM) mode yields higher selectivity and much higher sensitivity than in scan mode because the mass detector spends more time in each measurement cycle collecting ions at a specified mass/charge (*m/z*) ratio. A signal in SIM mode can be used to monitor a number of discrete masses for greater flexibility. The coeluting peaks can be separated by appropriate use of SIM signals or by extracted ion chromatograms (EIC) for individual ion *m/z* values. In this case, *m/z* 207 and 233 signify isoproturon and diuron (Figure 2). The expanded section of the TIC chromatogram overlaid with the extracted ion chromatograms in Figure 3 allows closer examination of the coeluting compounds. The selected mass spectra of diuron and isoproturon are shown in Figures 4a and 4b. The spectrum of diuron (Figure 4a) shows a good resolution of the isotope pattern of this doubly chlorinated compound and allows clear differentiation from the isotopic pattern of the non-chlorinated coeluting compound isoproturon (Figure 4b).

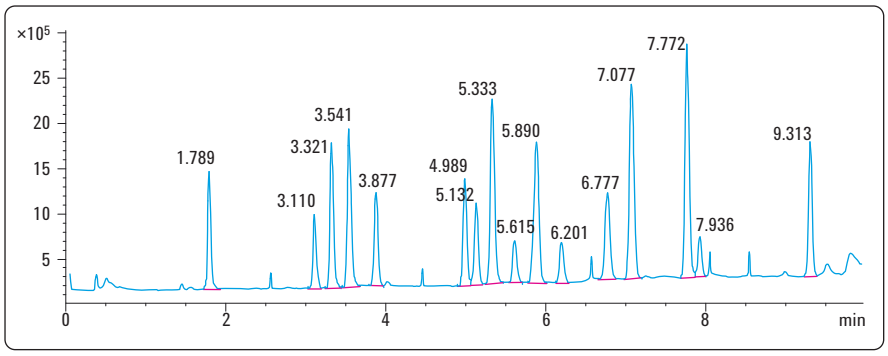


Figure 1
Chromatogram of the pesticide mixture.

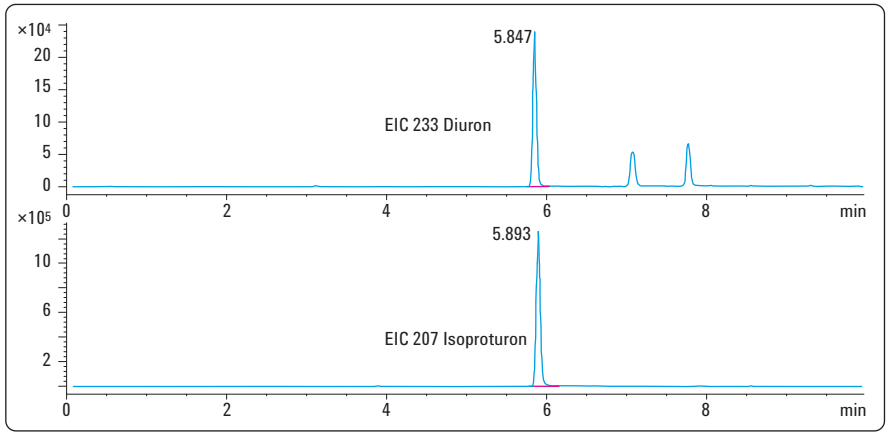


Figure 2
Extracted ion chromatograms EIC 233 and EIC 207 for the pesticide mixture.

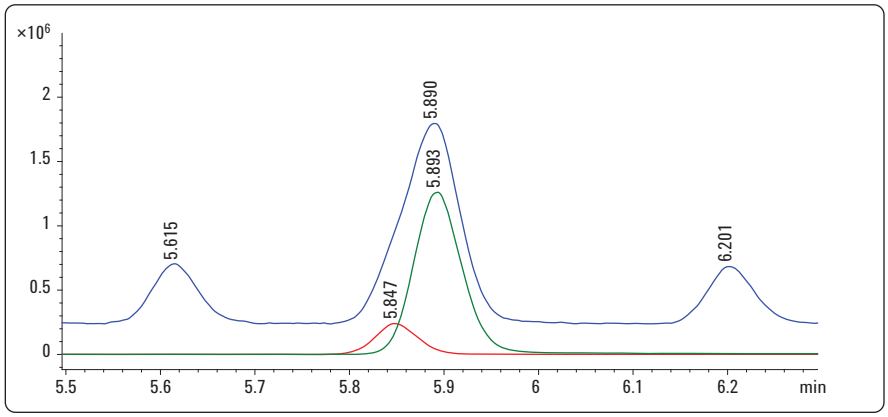


Figure 3
Detail view of coeluting diuron and isoproturon peaks showing the scan TIC and extracted ion chromatograms overlaid.

Peak Number	Component	Retention (mins)	m/z [M+H]+
1	Atrazine-desethyl	1.791	188
2	Metoxuron	3.111	229
3	Hexazinone	3.323	253
4	Simazine	3.542	202
5	Cyanazine	3.878	241
6	Methabenzthiazuron	4.990	222
7	Chlorotoluron	5.133	213
8	Atrazine	5.336	216
9	Monolinuron	5.616	215
10*	Diuron	5.847	233
11*	Isoproturon	5.893	207
12	Metobromuron	6.203	259
13	Metazachlor	6.777	278
14	Sebuthylazine	7.080	230
15	Terbuthylazine	7.773	230
16	Linuron	7.936	249
17	Metolachlor	9.313	284

* Diuron and isoproturon coelute

Table 2
Peak assignments.

Development of a fast method using a 100 mm column

Agilent ZORBAX C18 RRHD 1.8 µm stationary phase material offers high efficiency separations and supports faster flow rates due to the flatter Van Deemter profile associated with the small particle size. The 10-min method was converted to a 5-min method by doubling the flow rate to 1.0 mL/min and adjusting the gradient time (halving it) to keep the same gradient steepness in terms of the number of column volumes passing through the column during the gradient (Figure 5).

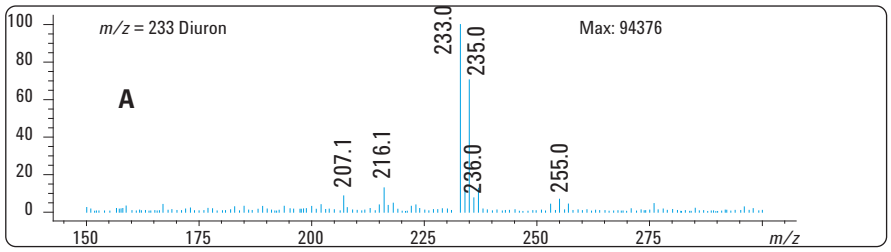


Figure 4a
Mass Spectrum of Diuron.

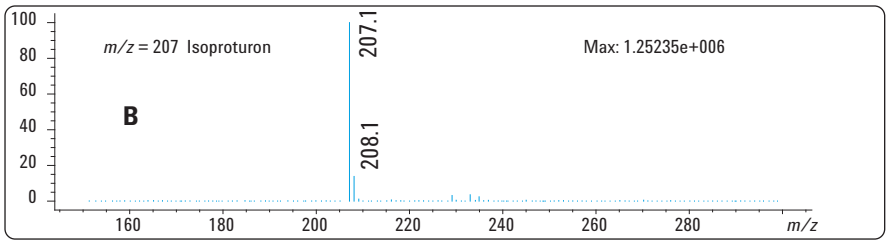


Figure 4b
Mass Spectrum of Isoproturon.

Agilent 1290 Infinity LC/UV Conditions				
Column 1	Agilent ZORBAX RRHD SB-C18 2.1 mm × 100 mm, 1.8 µm			
Column 2	Agilent ZORBAX RRHD SB-C18 2.1 mm × 50 mm, 1.8 µm			
Column Temperature	40 °C			
Mobile Phase A	Water + 0.1% formic acid			
Mobile Phase B	Acetonitrile + 0.1% formic acid			
Chromatogram shown in:	**Figure 1**	**Figure 5**	**Figure 7**	**Figure 8**
Column Length	100 mm	100 mm	50 mm	50 mm
Flow Rate	0.5 mL/min	1.0 mL/min	0.5 mL/min	1.0 mL/min
Mobile Phase Gradient:				
20% B	0.00 min	0.00 min	0.00 min	0.00 min
40% B	6.50 min	3.25 min	3.25 min	1.63 min
70% B	10.00 min	5.00 min	5.00 min	2.50 min
Injection Volume	10 µL	10 µL	5 µL	5 µL
Injection Needle Wash	In Flush Port, 10 s, acetonitrile/water (50/50)			
Diode-array Detector Signal A	242 nm, bandwidth 4 nm. Reference Off.			
Diode-array Detector Signal B	226 nm, bandwidth 4 nm. Reference Off			
Spectrograph Slit Width	4 nm			
Agilent 6140 Single Quadrupole MS Detector Conditions				
Ion source	Atmospheric pressure electrospray (API-ESI)			
Ion polarity	Positive			
Capillary Voltage	4000 V			
Drying gas flow	12 L/min			
Drying gas temperature	350 °C			
Nebulizer pressure	30 psi @ 0.5 mL/min; 50 psi @ 1.0 mL/min			
MSD Signal 1	Scan *m/z* 150 – 350			
MSD Signal 2	SIM *m/z* 188, 202, 207, 216, 222, 229, 249, 278 (5 ms dwell each)			
MSD Signal 3	SIM *m/z* 213, 215, 230, 233, 241, 253, 259, 284 (5 ms dwell each)			
Fragmentor voltage	90 V, all signals			

Table 3
Agilent 1290 Infinity LC/UV conditions.

The relationship between gradient steepness and flow is shown in the following equation

$$Gradient\ Steepness = \frac{Increase\ in\ stronger\ solvent}{Number\ of\ column\ volumes}$$

$$Gradient\ Steepness = \frac{\Delta\%B \cdot V_M}{F \cdot t_G}$$

where:

Δ%B is the range of the stronger mobile phase component across the gradient

V_M is the delay volume of the column, or the volume of mobile phase in the column

F is the flow rate

t_G is the time range (duration) of the gradient.

This is linked to the concept of gradient retention factor, k* in the following equation, and illustrates how a constant gradient steepness keeps the relative spacing of the peaks constant:

$$k^* = \frac{t_G}{\Delta\%B} \cdot \frac{F}{V_M} \cdot \frac{100}{S}$$

S is a compound-specific factor for each solute.

As expected, the overall appearance of the chromatogram is the same but the retention times are half of the original. Closer examination reveals some slight selectivity changes. The diuron and isoproturon coelution peak at 2.9 minutes shows a distinct shoulder indicating slightly more separation between these two compounds. Peaks 6 and 7 now slightly overlap (compare to Figure 1).

Because the gradient slope remained constant, it is concluded that frictional heating is generated from the flow rate and pressure. The separation was run at temperatures ranging from 30 to 45 °C to test the effect of temperature variation. As expected, the general

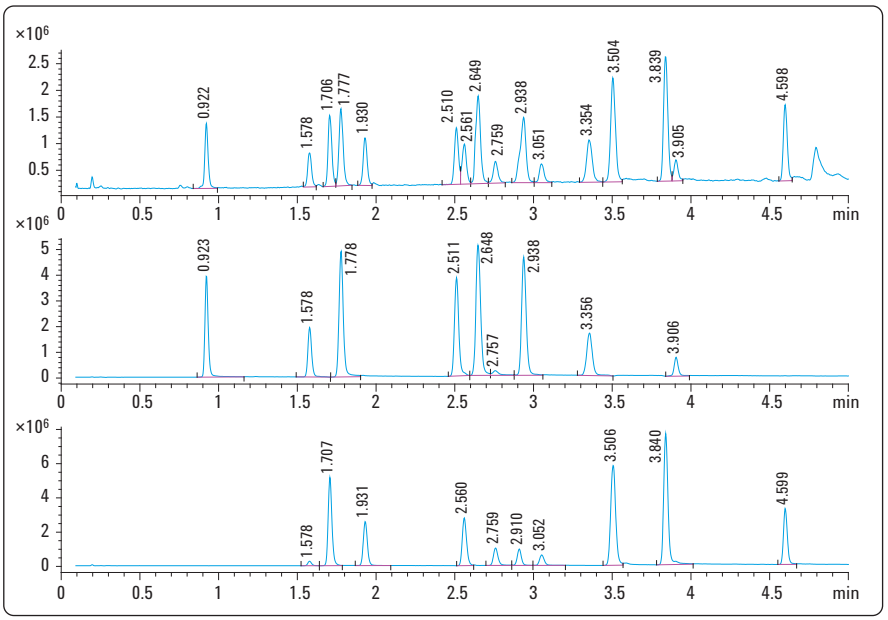


Figure 5
Pesticide mixture separated on 100 mm column in a 5 min gradient at 1 mL/min flow rate.

trend was to reduce retention as temperature increased. It can also be seen that the selectivity between various peak pairs is temperature sensitive. The following changes in selectivity were noted as the temperature increased: methabenzthiazuron and chlortoluron (peaks 6 and 7) decreased; chlortoluron and atrazine (peaks 7 and 8) increased; diuron and isoproturon (peaks 10 and 11) increased; and terbuthylazine and linuron (peaks 15 and 16) decreased.

The selectivity change observed in changing the flow rate and pressure may be indicated by a frictional heating effect. This can be estimated by empirical comparison at 1 to 2 °C. In this case the effect could be offset by lowering the thermostat temperature by 1-2 °C as reported elsewhere[1].

Frictional heating can cause band broadening if a radial temperature gradient is created in the column. This is influenced by the type of thermostatting employed in the system. The design of the 1290 Infinity LC column compartment takes care to avoid this. Therefore no extra dispersion was indicated by the observed peak widths.

Increasing the temperature, which reduces the viscosity of the mobile phase causes an observed difference in pressure readings (for example, 1110 bar at 30 °C and 920 bar at 45 °C). This effect is often used to create more headroom for pressure and flow rate increases (Figure 6).

Development of a fast method using a 50 mm column

Agilent ZORBAX C18 RRHD 1.8 µm columns offer high efficiency separations. Examination of the 100 mm column resolution data suggests that the separation is also adequate on a 50 mm column with the same packing material. Transferring a method from a 100 mm column to a 50 mm column follows the same approach as discussed above. Because the stationary phase is identical, the separation should be the same as long as the gradient is the same. The flow rate remains the same and because the column is half the length the peaks will elute in half the time. To maintain gradient slope, the time steps (t_G) in the timetable are divided by 2 because the column volume (V_M) is divided by 2. The 10-min method on the 100 mm column is reduced to a 5-min method on the 50 mm column.

The resulting chromatogram is shown in Figure 7. It is similar to the chromatogram shown in Figure 1 for the 100 mm column. It can be expected that the efficiency of the column measured in the number of theoretical plates (N) under isocratic conditions will be reduced by a factor of 2 for the 50 mm column compared to the 100 mm column. The effect on resolution is a factor of about 1.5 and it can be seen from the experimental results in Table 4 that peak width, resolution and peak capacity number are reduced by this factor. The most critical resolution for peaks separated in the scan chromatogram is between peaks 14 and 15 with a resolution of 1.2. However, the two peaks are totally separated by use of the SIM signals. The maximum separation pressure reached in the separation using the 50 mm column was 290 bar compared with 550 bar for the 100 mm column. Although 290 bar is attainable on a conventional (400 bar) HPLC system such as the Agilent 1200 HPLC system, the increased system delay volume intro-

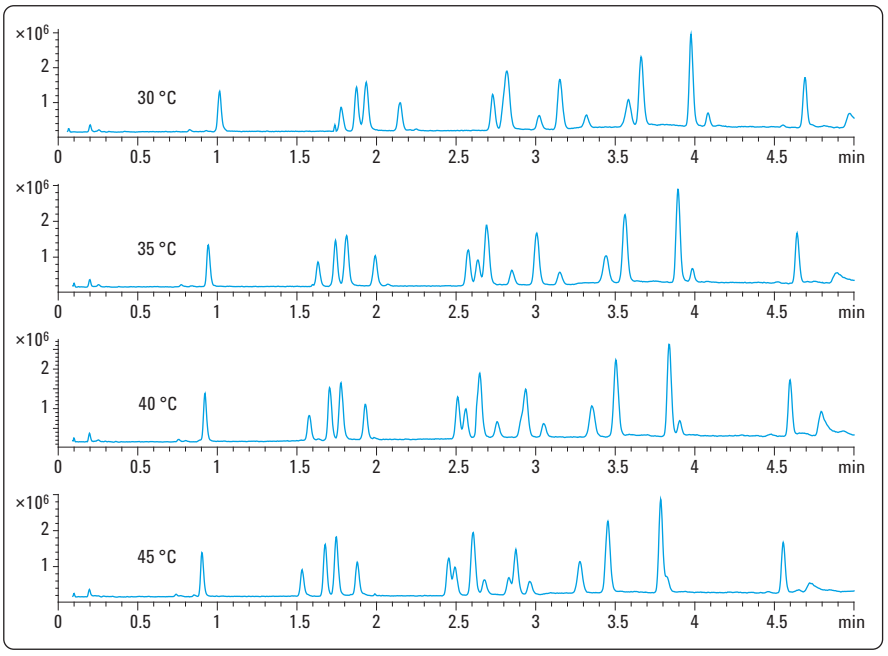


Figure 6
Effect of temperature on the separation: Chromatograms at 30°C (pressure, ΔP = 1110 bar), 35°C (ΔP = 1045 bar), 40°C (ΔP = 970 bar), and 45°C (ΔP = 920 bar).

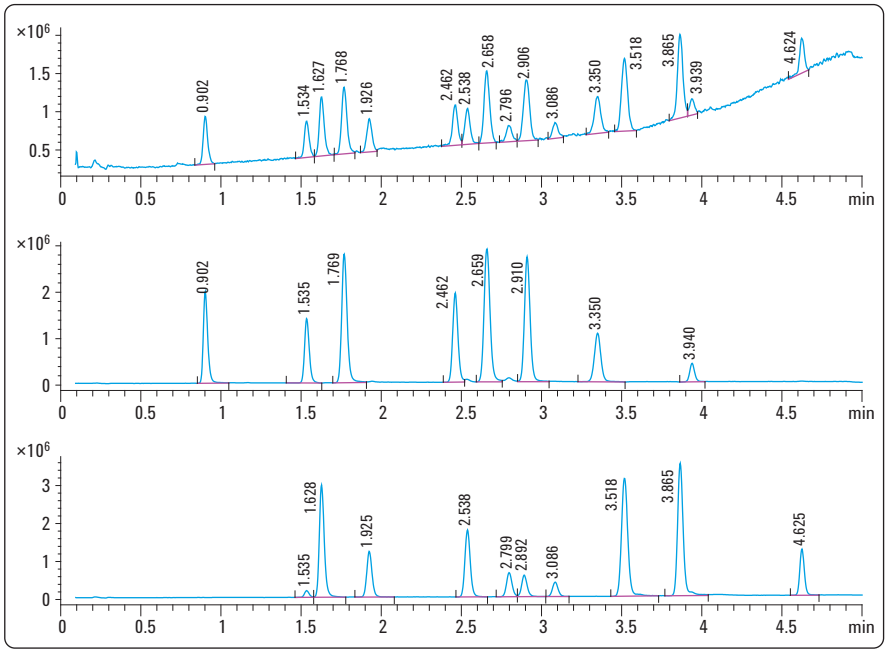


Figure 7
Pesticide mixture separated on 50 mm column in a 5 min gradient at 0.5 mL/min flow rate.

duces a longer isocratic delay at the start of the gradient. In addition, increased dispersion in the larger flow cell reduces resolution.

As with the 100 mm column, the same technique of increasing the flow rate and reducing the gradient times to maintain gradient slope was employed. Figure 8 shows the chromatogram at 1 mL/min and 2.5 minutes gradient for the 50 mm column with an observed maximum pressure of 550 bar. While the scan TIC chromatogram shows some loss of resolution, the SIM chromatograms still give good separation for all peaks. The maximum pressure (1200 bar) of the Agilent 1290 Infinity LC allows the flow rate to be increased by a factor of two. The electrospray source has a maximum flow rate of 1 mL/min, however, so additionial increases require the use of a flow splitter to maintain good MS detection.

	100 mm column, 10min gradient, 0.5 ml/min				50 mm column, 5 min gradient, 0.5 ml/min			
Peak	Ret Time	Width	Resol-ution	Peak Capacity	Ret Time	Width	Resol-ution	Peak Capacity
1	1.789	0.043		173	0.902	0.030		123
2	3.110	0.046	18.0	163	1.534	0.033	12.0	114
3	3.321	0.046	2.7	162	1.627	0.033	1.7	112
4	3.541	0.053	2.6	142	1.768	0.037	2.4	99
5	3.877	0.050	3.8	150	1.926	0.035	2.6	107
6	4.989	0.053	12.7	141	2.462	0.036	9.0	102
7	5.132	0.051	1.6	147	2.538	0.035	1.3	107
8	5.333	0.054	2.2	138	2.658	0.038	1.9	99
9	5.615	0.050	3.0	152	2.796	0.040	2.1	92
10	5.890	0.064	2.7	117	2.906	0.044	1.6	85
11	6.201	0.049	3.0	154	3.086	0.037	2.6	101
12	6.777	0.065	5.7	116	3.350	0.043	3.9	86
13	7.077	0.062	2.8	121	3.518	0.041	2.3	91
14	7.772	0.055	7.0	136	3.865	0.037	5.2	101
15	7.936	0.051	1.8	146	3.939	0.034	1.2	109
16	9.313	0.047	16.3	159	4.624	0.033	11.8	113

Table 4
Comparison of peak parameters on 100 mm and 50 mm columns.

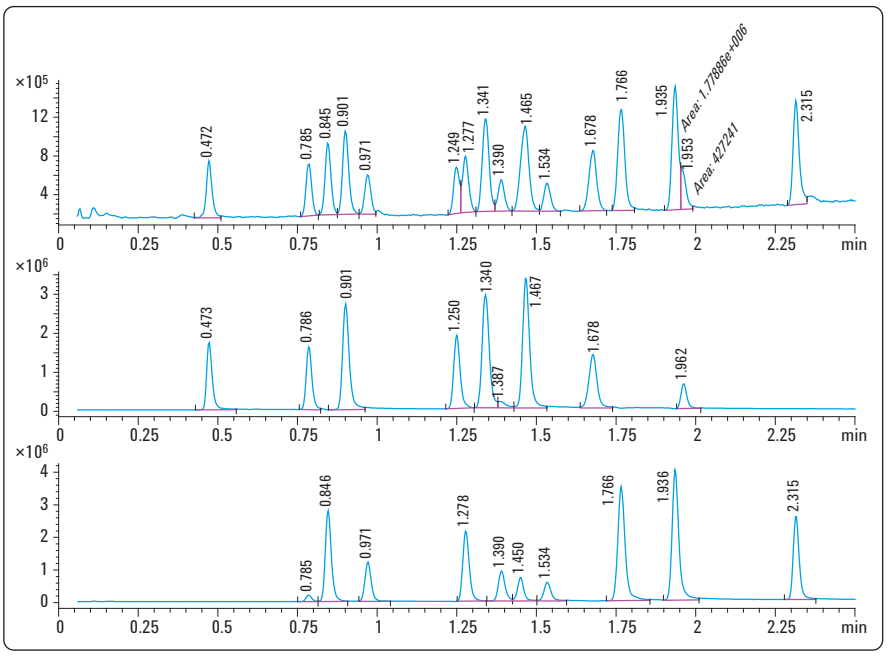


Figure 8
Pesticide mixture separated on 50 mm column in 2.5 minutes gradient at 1 ml/min flow rate.

Conclusion

This Application Note shows a method for the separation of a mixture of common herbicides from the triazine, phenylurea and chloroacetanilide classes in less than 10 minutes using a high efficiency sub-2-µm column with the Agilent 1290 Infinity LC system and Agilent 6140 Single Quadrupole LC/MS system. Several aspects of UHPLC method development, including frictional heating effects, are highlighted. Using normal method transfer techniques it is shown that this separation using MS detection can be accelerated to complete in less than 2.5 minutes.

Reference

1. Pat Sandra, "Increasing productivity in the analysis of impurities in meto clopramide hydrochloride formula tions using the Agilent 1290 Infinity LC system", Agilent Application Note 5990-3981EN, 2009.

www.agilent.com/chem/1290

May 1, 2010
Publication Number 5990-5794EN

Agilent Technologies