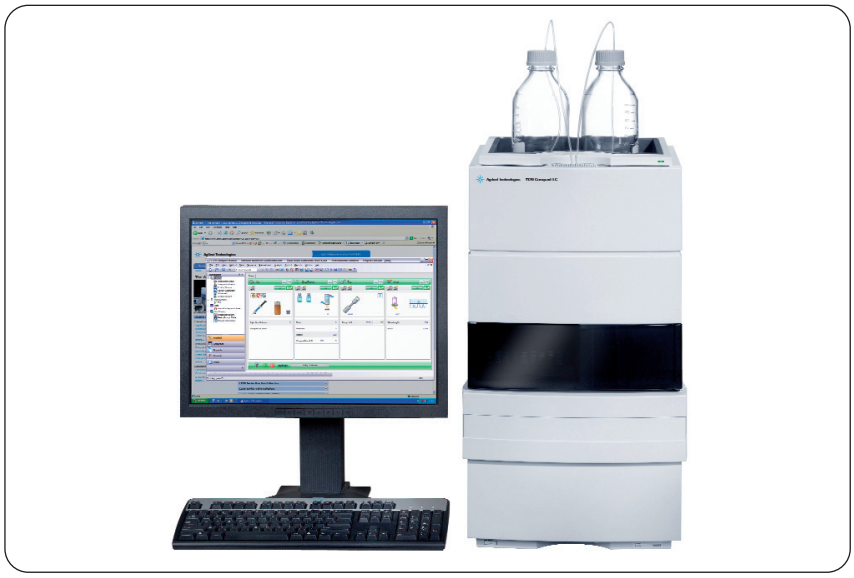
Performance characteristics of the Agilent 1120 Compact LC

An integrated LC system for optimum performance in conventional liquid chromatography

Technical Overview



Abstract

The Agilent 1120 Compact LC is the system of choice for conventional, analytical scale liquid chromatography. It is an integrated LC designed for ease of use, performance and reliability. The Agilent 1120 Compact LC is best suited for the analysis of a wide range of applications for the chemical, environmental, food and pharmaceutical industries. Instrument control is through Agilent EZChrom Elite Compact software. The Agilent 1120 Compact LC guarantees:

- High precision for retention times and areas
- Precise gradient formation
- Excellent signal-to-noise ratio

Agilent Equipment:

- 1120 Compact LC
- EZChrom Elite Compact software

Application Area:

- Environmental analysis
- Fine chemical manufacturing
- Food quality control
- Hydrocarbon processing
- Pharmaceutical analysis

Agilent Technologies

Introduction

The Agilent 1120 Compact LC is available in five different configurations. All configurations include Agilent EZChrom Elite Compact software for instrument control, data analysis and reporting, and Agilent Lab Monitor and Diagnostics software. The main area of application is conventional HPLC using 3 to 4.6 mm ID columns and flow rates greater than 0.5 mL/min.

This publication describes the performance characteristics of the Agilent 1120 Compact LC, for example:

- Precision of retention times and areas
- Gradient performance
- Detector noise and drift behavior

The tests described involved the analysis of different compound mixtures as well as the use of different gradients and solvents.

Experimental

Equipment

- Agilent 1120 Compact LC comprising gradient pump with integrated degasser, autosampler with vial tray, column oven and variable wavelength detector, (figure 1)
- Agilent EZChrom Elite Compact software

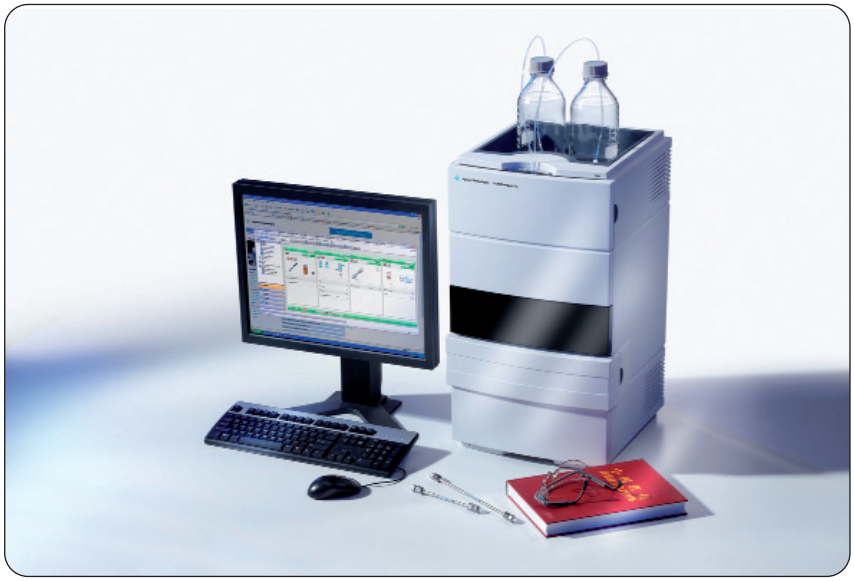


Figure 1
Agilent 1120 Compact LC.

Results and discussion

Key measurements for performance characterization

Several key measurements are necessary to evaluate the performance of an HPLC system. Some characteristics are influenced by only one part of the system. For example, linearity and detection limits are influenced mainly by the detector, whereas delay volume and composition accuracy are influenced by the pump, and carryover by the autosampler. In contrast, other characteristics such as baseline noise and precision of retention times and peak areas are influenced by the entire system. This publication describes the following key measurements:

- Pump – composition accuracy, precision, ripple, precision of retention times, delay volume
- Autosampler – precision of peak areas, linearity, carryover
- Detector – baseline noise, drift, wander, linearity, sensitivity

Although certain measurements are assigned to a particular module in this list, the results could be influenced by several modules.

Pump performance

Delay volume and composition accuracy

Delay volume is defined as the volume between the point of solvent mixing and the column. Parameters affecting delay volume include the pump, mixers, injectors and tubing and fittings that connect the modules of the system. Large delay volumes affect the sharpness of the gradient and therefore the selectivity of an analysis. They also increase the

run-time cycle, especially at low flow rates. The delay volume depends on the backpressure and was measured by running a tracer gradient. The delay volume for an Agilent 1120 Compact LC is approximately 850 µL. Figure 2 shows the step gradient from 0 to 100 % tracer in steps of 10 % which was used to evaluate gradient accuracy. In figure 2 the signal trace and programmed step gradient are superimposed and demonstrate excellent conformance between the two traces.

Gradient precision and mixing noise

To achieve highly reproducible separations based on gradient runs it is important that the pump mixes the solvents accurately and precisely. A step gradient from 0 to 100 % tracer with water was used with a water/acetone tracer. The detector signal at 263 nm was used to monitor the precision and mixing noise of the pump. A restriction capillary was used instead of a column. Figure 3 shows three consecutive step gradients superimposed and proves that gradients can be run with high precision. The mixing noise was calculated as the peak-to-peak noise of each step expressed in units of %B. Over the complete range the noise was typically less than 0.05 % related to the 100 % step.

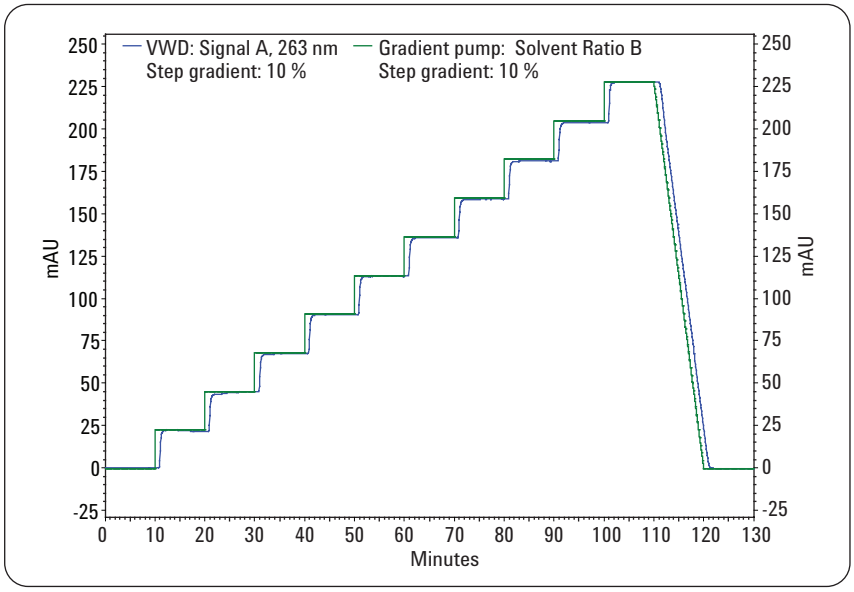


Figure 2
Step gradient to determine pump delay volume, mixing noise, accuracy.

Chromatographic conditions:

Column:	Restriction capillary
Mobile phase:	A: Water, B: Water + 0.1 % acetone
Gradient:	0 to 100 %B in 10 % steps
	Steps were held for 10 min
Flow rate:	1 mL/min
Injection volume:	0 µL
Column temperature:	36 °C
Detection:	263 nm, peak width > 0.025 min, data rate 20 Hz

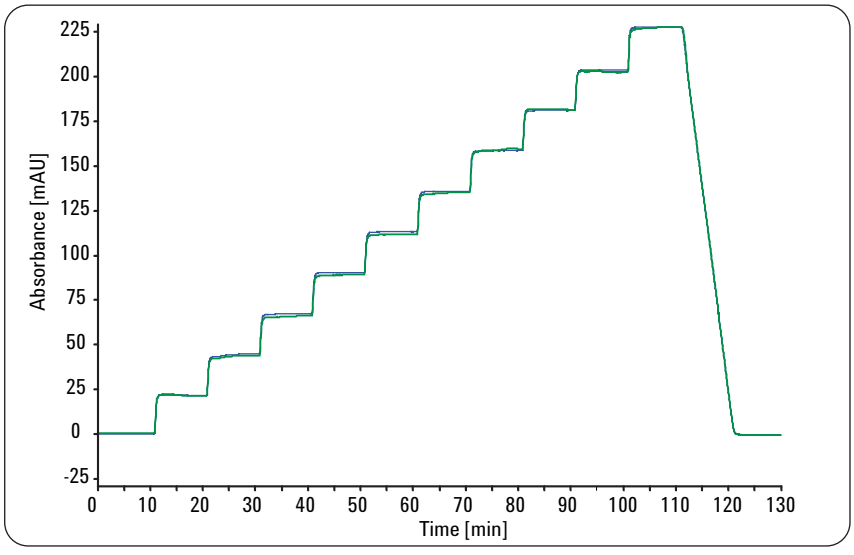


Figure 3
Overlay of three step gradient runs from 0 to 100 % tracer in 10 % steps to determine precision.

Performance of linear gradient

Gradients are frequently used today and need to be accurate and precise to be able to produce consistent data. In figure 4 the programmed gradient is superimposed on the linear gradient run from 5 to 95 %B in 20 minutes, demonstrating the excellent accuracy of the pump. The three linear gradient runs shown in figure 5 demonstrate that the precision of the pump is also excellent.

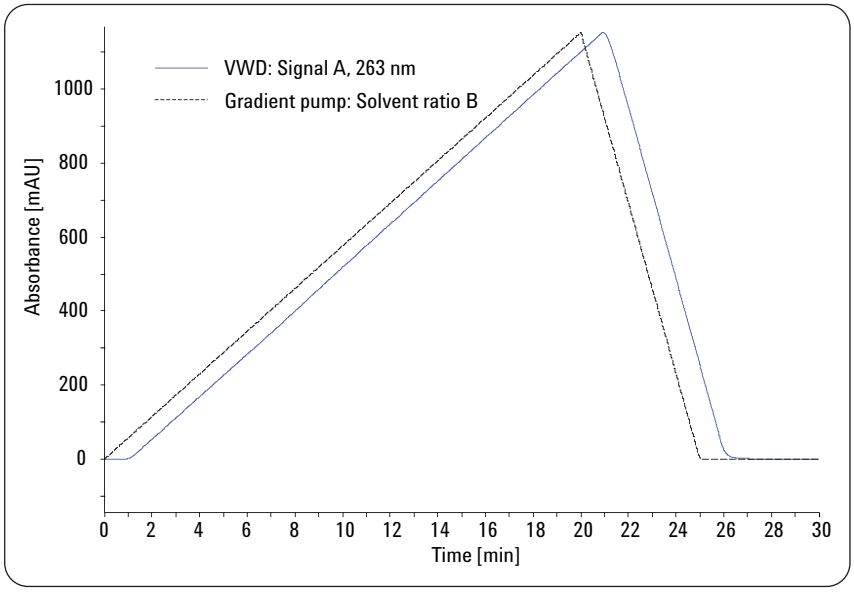


Figure 4
Overlay of programmed gradient and linear gradient run.

Chromatographic conditions:

Column:	Restriction capillary
Mobile phase:	A: Water, B: Water + 0.5 % acetone
Gradient:	5 to 95 % tracer in 20 min
Flow rate:	1 mL/min
Injection volume:	0 µL
Column temperature:	36 °C
Detection:	263 nm, peak width > 0.025 min, data rate 20 Hz

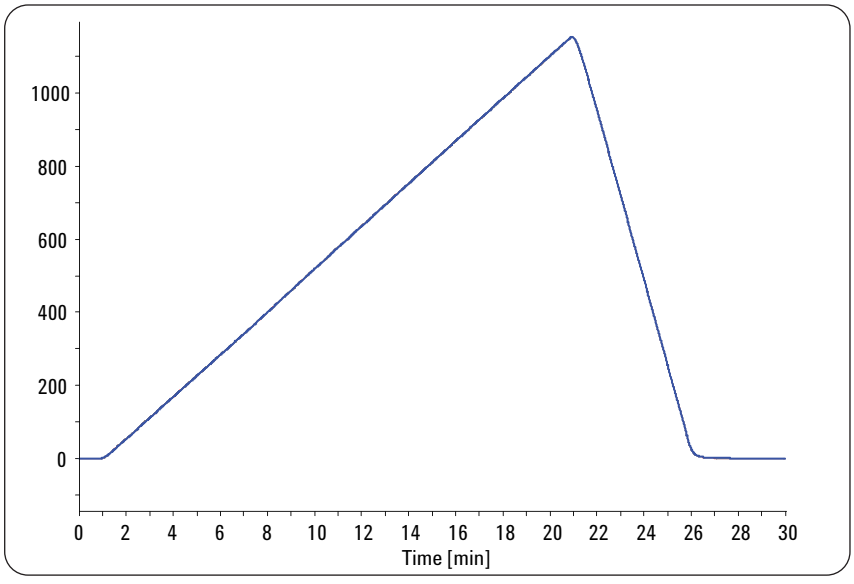


Figure 5
Overlay of three consecutive linear gradient runs.

Precision of retention times

Retention time precision is an important characteristic that influences qualitative and quantitative results. The precision depends on the performance of the pump and degasser, and on the stability of the column temperature. To measure precision, a standard sample was injected several times and the relative standard deviations of retention times were calculated. The pump showed excellent precision of retention times. Chromatograms of the evaluated sample are shown in figures 6 and 7 for the isocratic and gradient analyses respectively. For the isocratic analysis the retention time precision was less than 0.1 % RSD and for the gradient analysis the precision was less than 0.05 % RSD.

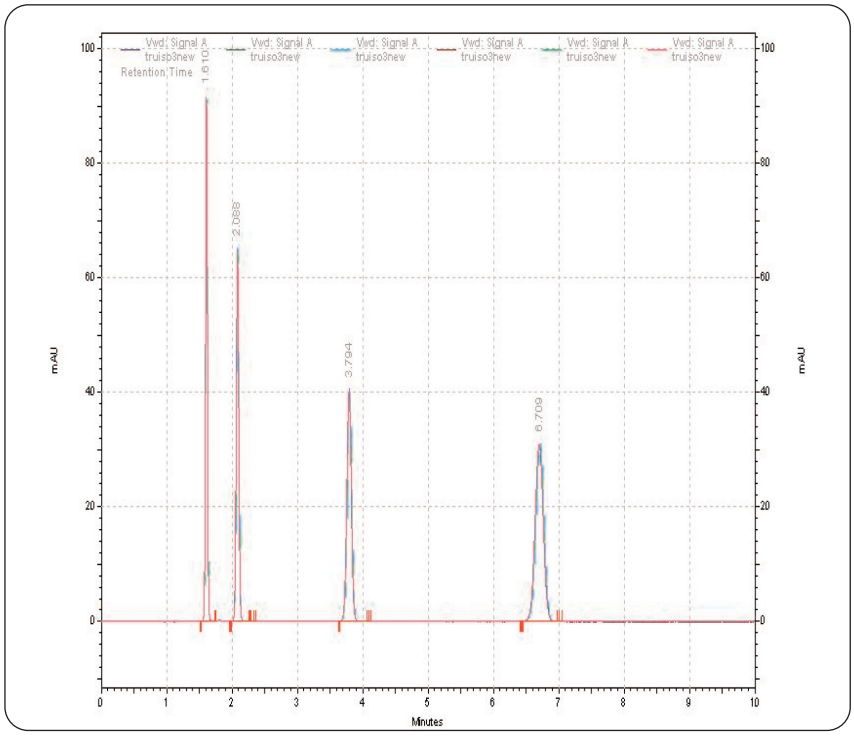


Figure 6
Isocratic analysis for determination of retention time precision.

Chromatographic conditions:

Sample:	Isocratic standard sample (dimethylphthalate, diethylphthalate, biphenyl, o-terphenyl) diluted 1:5
Column:	Agilent HC-C18(2), 4.6 x 150 mm, 5 µm
Mobile phase:	Water/acetonitrile, 30:70
Flow rate:	1.5 mL/min
Injection volume:	3 µL
Column temperature:	40 °C
Detection:	254 nm, peak width > 0.0025 min, response time 0.06 s

Chromatographic conditions:

Sample:	1. Vitamin C 2. Propyl gallat (PG) 3. 2,4,5-trihydroxy-butyrophenone (THBP) 4. Mono-tert-butyl-hydroquinone (TBHQ) 5. Butylated hydroxyanisole (BHA) 6. 4-hydroxymethyl-2.6-di(tert-butyl)phenol (ionox 100) 7. Butylated-hydroxytoluene (BHT) 8. Ascorbyl-palmitate (ACP)
Column:	Agilent HC-C18(2), 4.6 x 150 mm, 5 µm
Mobile phase:	A: Water + 0.05 % TFA B: Acetonitrile + 0.045 % TFA
Gradient:	10 to 95 %B in 15 min
Flow rate:	1.5 mL/min
Injection volume:	5 µL
Column temperature:	40 °C
Detection:	260 nm, peak width > 0.0025 min, response time 0.06 s

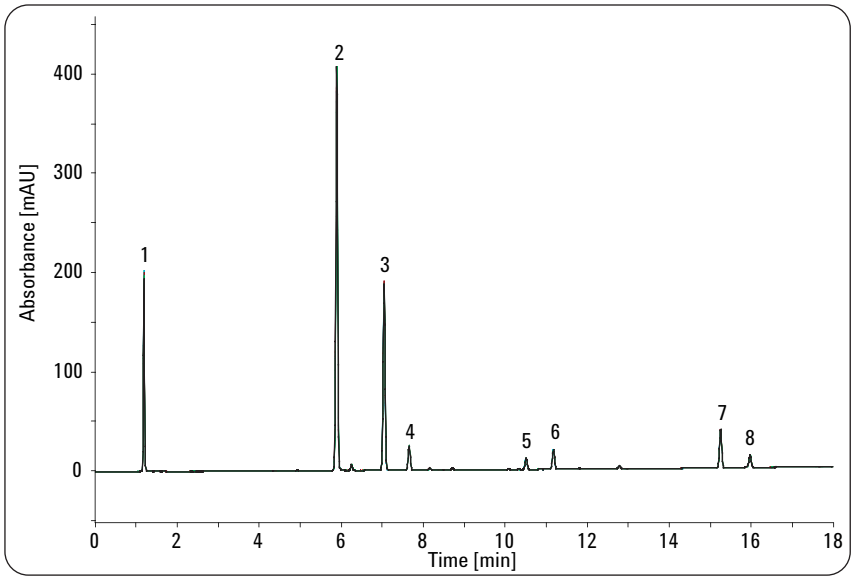


Figure 7
Gradient analysis for determination of retention time precision.

Autosampler performance

Precision of peak areas

Peak area precision depends on the injection volume and is an important characteristic that influences quantification. The precision deteriorates at low injection volumes and therefore should be measured at different volumes. Injection volumes depend on the concentration of samples. A wider injection volume range of an autosampler is better for different sample concentrations. For a high performance autosampler it is important to show good precision over the complete injection volume range. For the Agilent 1120 Compact LC autosampler an injection volume range from 0.1 up to 100 µL is possible without the need to modify the hardware. The area precision was tested using herbicides as sample compounds (figure 8). Table 2 summarizes the precision data for the peak areas.

Linearity

The linearity of the injector was measured from 1 to 80 µL and the results are shown in figures 9. The data demonstrate the excellent injection volume linearity of the autosampler. A caffeine standard was used at a concentration of 25 µg/mL. The tested injection volumes were 1, 3, 5, 10, 40 and 80 µL. The analysis was run under isocratic conditions. For the tested range the coefficient of correlation was 0.999998.

Chromatographic conditions:

Sample:	Caffeine standard, 25 µg/mL (Agilent part # 8500-6762)
Column:	Agilent ZORBAX SB C18, 3 x 100 mm, 3.5 µm
Mobile phase:	Water/acetonitrile, 85:15
Flow rate:	0.8 mL/min
Column temperature:	36 °C
Detection:	272 nm, peak width > 0.05 min, data rate 10 Hz

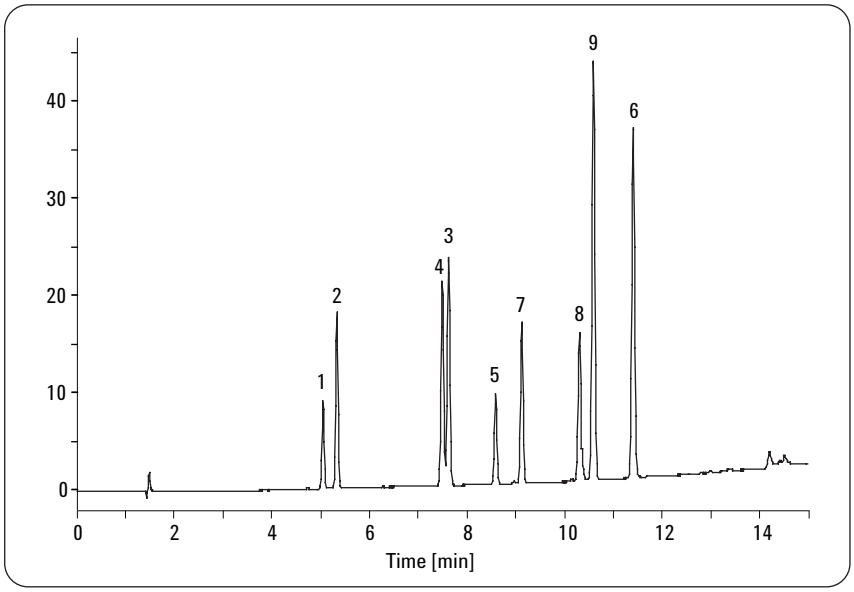


Figure 8
Analysis of herbicides for the determination of peak area precision.

Chromatographic conditions:

Column:	Agilent HC-C18 (2), 4.6 x 150 mm, 5 µm
Mobile phase:	A: Water, B: Acetonitrile
Gradient:	10 to 90 %B in 15 min
Flow rate:	1.5 mL/min
Injection volume:	5 µL
Column temperature:	40 °C
Detection:	225 nm, peak width > 0.0025 min, response time 0.06 s

Peak	Compound	1:100 dilution 5 µL inj. vol. (ng per inj.)	RSD Area
1	Metamitron	16.5	0.19
2	Chloridazone	12.5	0.17
3	Simazine	12.5	0.12
4	Cyanazine	18.5	0.10
5	Prometryn	24	0.39
6	Chlortoluron	48	0.26
7	Diuron	10	0.34
8	Propazine	23	0.16
9	Terbuthylazine	29	0.33

Table 1
Analyzed herbicides and concentration used.

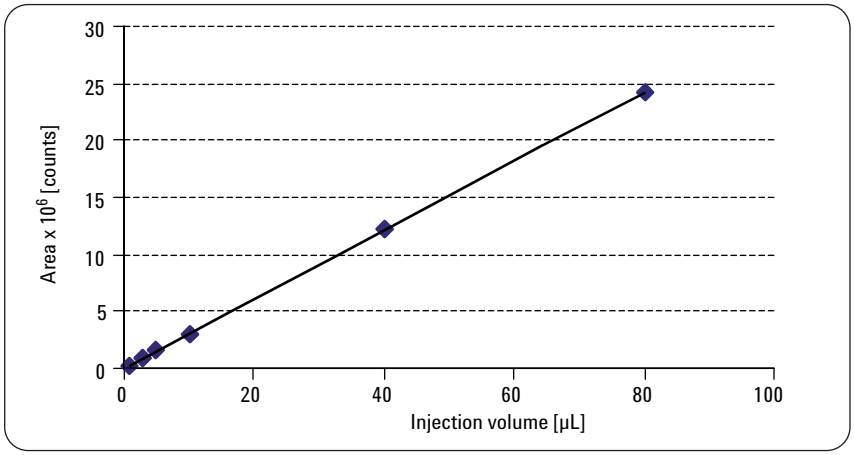


Figure 9
Injection volume linearity greater than 0.99999.

Carryover

Peak area precision also can deteriorate when non-reproducible carryover of samples occurs. The flow-through design of the Agilent 1120 Compact LC autosampler prevents carryover to a large extent. All parts of the injector that come in contact with the sample are flushed continuously with mobile phase throughout the entire run. It is also possible to wash the exterior of the needle in a separate wash vial or even in several vials. Carryover for the 1120 Compact LC was tested using caffeine as test compound. 2.5 µg in 5 µL were injected three times followed by an injection of 5 µL pure water. The carryover was less than 0.03 % (figure 10).

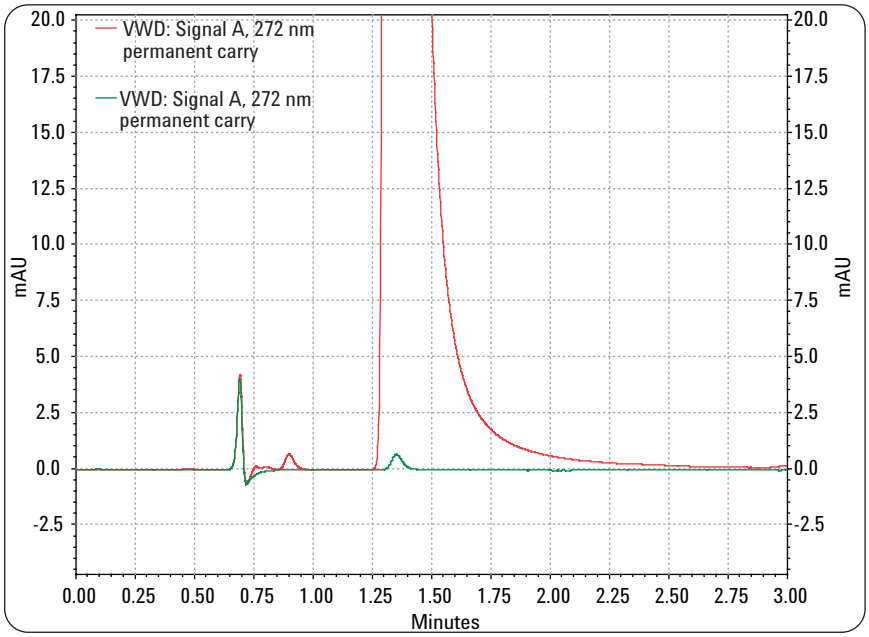


Figure 10
Less than 0.03 % carryover for caffeine as test compound

Chromatographic conditions:

Sample:	Caffeine standard 500 µg/mL, Agilent order number 8500-6762
Column:	Agilent ZORBAX SB C18, 100 x 3 mm, 3.5 µm
Mobile phase:	Water/acetonitrile, 85:15
Flow:	0.8 mL/min
Injection volume:	5 µL
Column temperature:	36 °C
Detection:	272 nm, peak width > 0.05 min, data rate 10 Hz

Detector performance

Short-term noise and drift

Short-term noise and drift are characteristics that determine the performance of an LC detector. Low noise is especially important when analyzing compounds at trace levels, whereas drift affects the integration quality. Detector noise and drift were determined according to ASTM (American Society for Testing and Materials) with the overall system noise using water as mobile phase. The noise determination was based on the *"ASTM E-1657-94: Standard practice for testing variable wavelength photometricdetectors used in liquid chromatography"*. Table 2 shows the results of the short-term noise and drift measurements for the variable wavelength detector.

Time [min]	ASTM noise [mAU]	Drift [mAU/h]
6–7.1	0.008	
7–8.1	0.003	
8–9.1	0.005	
9–10.1	0.004	
12–13.1	0.003	
19–20.1	0.003	
0–20		0.088

Table 2
Noise and drift performance of variable wavelength detector.

Chromatographic conditions:

Column:	Agilent ZORBAX SB C18, 100 x 3 mm, 3.5 µm
Mobile phase:	Water
Flow:	1 mL/min
Column temperature:	36 °C
Detection:	254 nm, peak width > 0.2 min, data rate 2.5 Hz

Chromatographic conditions:

Sample:	Caffeine standards
Column:	Agilent HC-C18(2), 4.6 x 150 mm, 5 µm
Mobile phase:	Water + 0.05 % TFA (trifluoro acetic acid)/acetonitrile + 0.045 % TFA, 85:15
Flow rate:	1.5 mL/min
Injection volume:	5 µL
Column temperature:	40 °C
Stop time:	5 min
Detection:	272 nm, peak width > 0.05 min, data rate 10 Hz

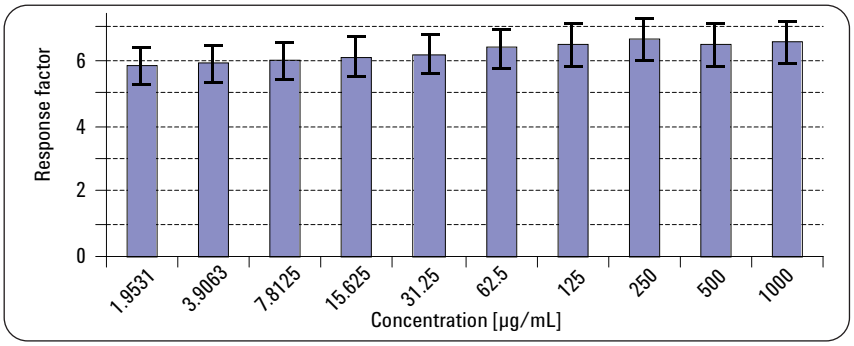


Figure 11
Detector linearity over the range from 5 to 2250 mAU.

Detector linearity

The linear range of a detector represents the range of concentration of a substance over which the sensitivity is constant within a specified variation, usually ± 5 %. Linearity is an important characteristic that influences quantification. The linearity also depends on the type of compound and on the wavelength setting. The detector linearity is determined by a series of standards with increasing concentrations that cover the range of interest. The detector linearity was measured by injecting caffeine standards with increasing concentrations over the range 5 mAU to 2200 AU. Figure 11 shows the linearity plot. This is a plot of detector response factor (concentration/area counts) against the concentration and covers the range 2–1000 µg/mL. The linear range of the detector is the concentration range in which the linearity plot lies between the horizontal lines 5 % above and below. The coefficient of correlation for areas and heights was better than 0.99995.

Limit of detection

The limit of detection was determined by injecting herbicides at trace levels in an injection volume of 20 µL. A data rate of 14 Hz was used. The limit of detection was calculated from the signal-to-noise ratio (peak-to-peak noise). At a data rate of 14 Hz the limit of detection was less than 150 pg. Table 3 summarizes the results for the determination of limit of detection. Figure 12 shows the standard chromatograms superimposed on chromatograms of a spiked water sample and a blank water sample. The standard in figure 12 represents the injected amounts listed in table 3.

Summary

The Agilent 1120 Compact LC provides excellent performance and quality of results for routine applications in the chemical, environmental, food and pharmaceutical industries.

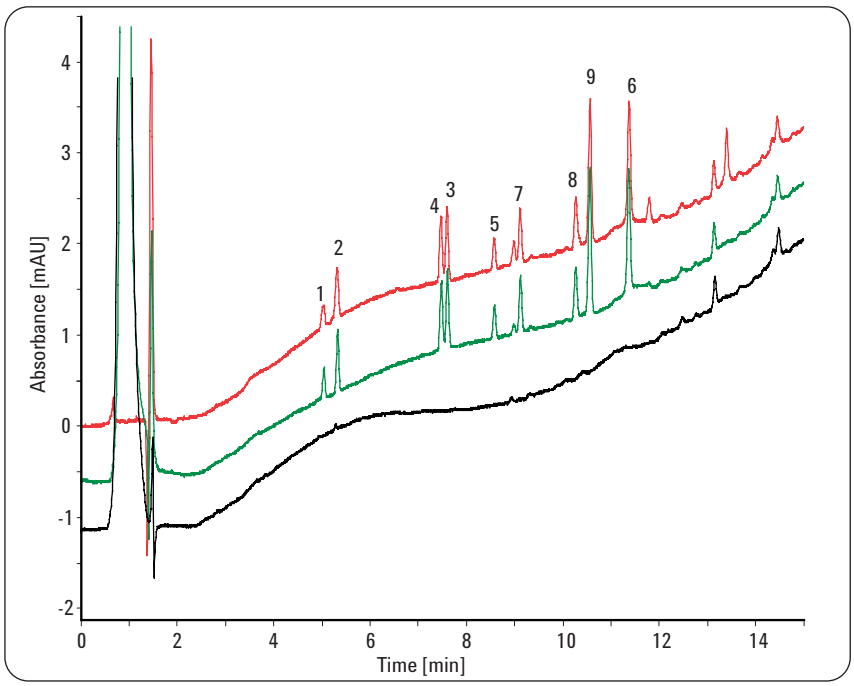


Figure 12
Overlay of standard with spiked water sample: Upper trace – 1:10,000 diluted stock solution, Center trace – spiked drinking water sample, Lower trace – blank drinking water sample

Chromatographic conditions:

Column:	Agilent HC-C18(2), 4.6 x 150 mm, 5 µm
Mobile phase:	Water/acetonitrile
Gradient:	10 to 90 %B in 15 min
Flow rate:	1.5 mL/min
Injection volume:	20 µL
Column temperature:	40 °C
Detection:	225 nm, peak width > 0.0025 min, response time 0.06 s

Peak	Compound	Injected amount of standard [ng]	LOD with S/N = 3 P/to/P noise 0.02 mAU [pg]
1	Metamitron	0.66	132
2	Chloridazone	0.5	50
3	Simazine	0.5	50
4	Cyanazine	0.74	49
5	Prometryn	0.96	144
6	Chlortoluron	1.920	128
7	Diuron	0.4	34
8	Propazine	0.92	34
9	Terbuthylazine	1.160	37

Table 3
LOD evaluation for herbicides.

- The isocratic and gradient pumps are highly accurate and precise for excellent retention time precision. The relative standard deviation for retention times is typically less than 0.1 %.
- The autosampler ensures highly reliable quantitative results. The relative standard deviation for areas is typically less than 0.5 %.
- The variable wavelength detector is best suited for sensitive analysis of compounds in the mid to low picogram range.

www.agilent.com/chem/1120

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