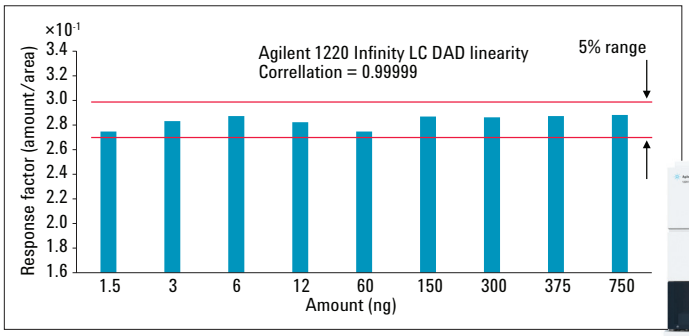
Performance Characteristics of the Agilent 1220 Infinity Gradient LC System with Diode Array Detector and Mobile Upgrade Kit

Technical Overview



Author

Sonja Schneider
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

The Agilent 1220 Infinity Gradient LC system is now available with a built-in diode array detector that offers a data acquisition rate of 80 Hz for full spectra. In combination with the Agilent 1220 Infinity Mobile Upgrade Kit, the system is resistant against shocks or vibrations during transportation in a mobile vehicle. The 1220 Infinity Mobile LC Solution is a robust and rugged system for onsite measurement. The performance of the system before and after vibration tests on a moving tray was evaluated with respect to retention time and area precision as well as noise and drift of the diode array detector. The performance of the diode array detector was analyzed in detail.

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Introduction

The 1220 Infinity Gradient LC system is an integrated, binary-gradient liquid chromatography system with a pressure range of up to 600 bar, supporting both HPLC and UHPLC technology, including sub-2 µm and superficially porous columns. The system is available with a built-in diode array detector (DAD) for multiwavelength detection and spectra analysis. The DAD features a data acquisition rate 80 Hz, multiwavelength detection and spectral analysis. Other system modules include a dual-channel gradient pump, autosampler and column oven. The gradient pump has a flow rate range from 0.2 to 10 mL/min (5 mL at 600 bar, 10 mL at 200 bar), low pressure mixing and an integrated degassing unit. The autosampler operates with an injection volume range from 0.1 to 100 µL and a capacity of one hundred 2-mL vials. The column oven holds one 25-cm column, and the maximum temperature is 60 °C.

The 1220 Infinity LC Mobile Upgrade Kit consists of functional parts that enable the 1220 Infinity LC system to be mounted in a mobile laboratory vehicle so it can be moved to different locations to access remote measurement sites. The main component is the attenuation unit that acts as a shock absorber to protect the instrument during transit or from influences of operators moving in the mobile laboratory. A solvent bottle unit keeps the bottles fixed to the instrument. Wire mesh keeps the column safe in the column oven, and the mobile solvent compartment secures the solvent bottles during operation.

This Technical Overview shows that a wide range of parameters were tested with detailed DAD performance evaluation.

The pump and autosampler were tested for:

- Precision of retention times
- Precision of areas

The DAD was tested for:

- ASTM drift and noise for the 10-mm path length cell
- Linearity over a wide range
- Limit of detection for anthracene for the 10-mm path length cell
- Spectral performance

As the 1220 Infinity Gradient LC System with DAD is a mobile solution, the robustness and ruggedness was tested regarding pump, autosampler, and detector performance (noise and drift) before and after vibration tests on a moving tray.

Experimental

Instrumentation

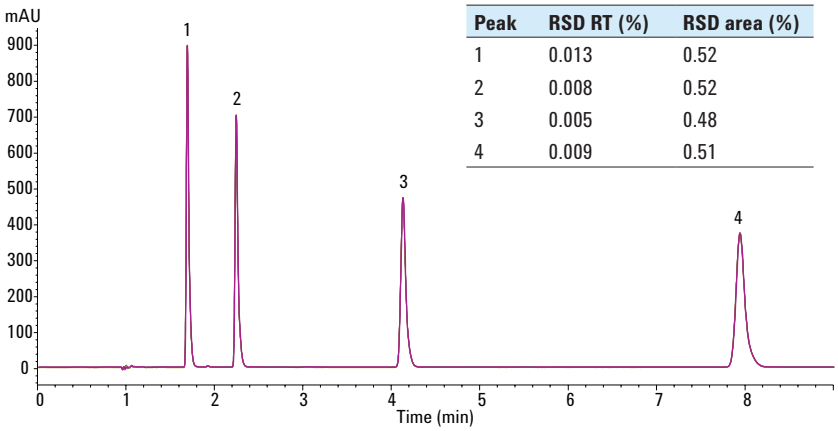
The Agilent 1220 Infinity Gradient LC System (G4294B) was equipped with a dual-channel gradient pump with integrated degassing unit, autosampler, column compartment, and the diode array detector. For transportation, the system was mounted on a transportation plate, 1220 Infinity Mobile Upgrade Kit (G4292A).

Software

- Agilent OpenLAB CDS ChemStation Edition for LC & LC MS Systems, Rev. C.01.04 [35]
- Agilent OpenLAB CDS 3D UV Add-On Software.

Pump and Autosampler Performance – Precision of Retention Times and Areas

Retention time precision was tested with different gradient and isocratic conditions using 4.6 and 3-mm id columns. The relative standard deviation (RSD) of retention times is typically < 0.2% for gradient analysis. Figure 1 shows an example of an isocratic application. The flow precision was < 0.015% RSD for the retention times.



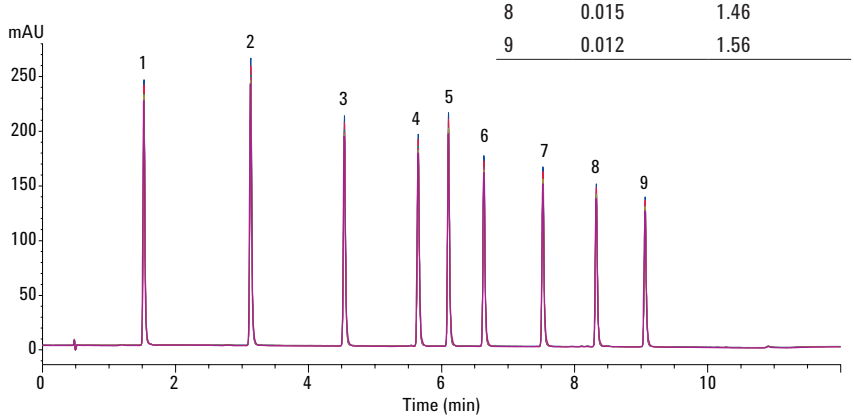
Peak	RSD RT (%)	RSD area (%)
1	0.013	0.52
2	0.008	0.52
3	0.005	0.48
4	0.009	0.51

Chromatographic conditions	
Sample:	Isocratic test sample (p/n 01080-68707)
Column:	Agilent ZORBAX RRHT, Eclipse Plus C18, 4.6 × 150 mm, 1.8 µm (p/n 959994-902)
Mobile phase:	A = Water B = Acetonitrile
Isocratic:	30/70 A/B
Flow rate:	1.2 mL/min
Stop time:	9 minutes
Injection volume:	5 µL, draw speed 200 µL/min
Column temperature:	40 °C
Detection:	254 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.025 minutes (0.5 second response time, 10 Hz)

Figure 1
Precision for an isocratic run at 230 bar with a retention time (RT) precision < 0.015% RSD.

Figure 2 shows an example of a gradient run. The retention time precision was < 0.063% RSD, except for the first peak.

Peak	RSD RT (%)	RSD area (%)
1	0.101	0.79
2	0.062	0.96
3	0.033	0.99
4	0.017	1.11
5	0.013	1.09
6	0.011	1.10
7	0.011	1.24
8	0.015	1.46
9	0.012	1.56

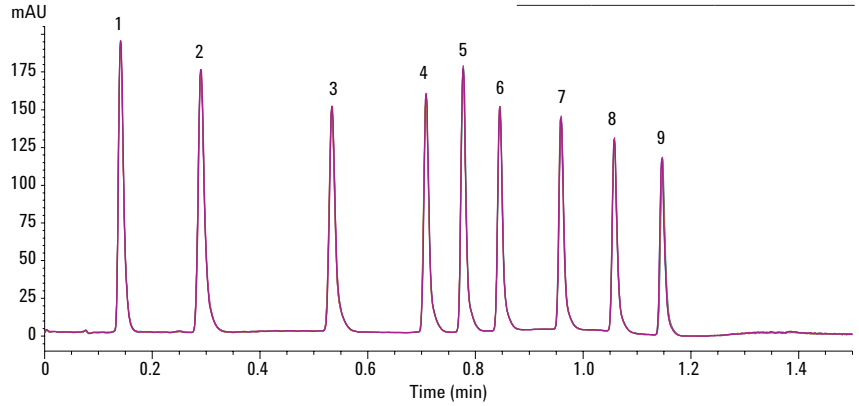


Chromatographic conditions	
Sample:	Agilent 1200 Series Rapid Resolution LC system checkout sample (p/n 5188-6529)
Column:	Agilent ZORBAX Solvent Saver HT, Eclipse Plus C18, 3.0 × 100 mm, 1.8 µm (p/n 959964-302)
Mobile phase:	A = Water B = Acetonitrile
Gradient:	20% B to 90% B in 10 minutes
Flow rate:	1.0 mL/min
Stop time:	12 minutes
Post-time:	5 minutes
Injection volume:	1 µL
Column temperature:	40 °C
Detection:	245 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.025 minutes (0.5 second response time, 10 Hz)

Figure 2
Precision for a gradient run at 434 bar with a RT precision < 0.063% RSD except for the first peak.

Figure 3 shows an example of a fast gradient run at 590 bar. In this experiment, an Agilent Poroshell 120 EC-C18 column was used. The retention time precision was < 0.08% RSD.

Peak	RSD RT (%)	RSD area (%)
1	0.045	0.86
2	0.078	0.43
3	0.056	0.51
4	0.028	0.45
5	0.028	0.74
6	0.023	0.48
7	0.037	0.66
8	0.016	0.73
9	0.048	0.77



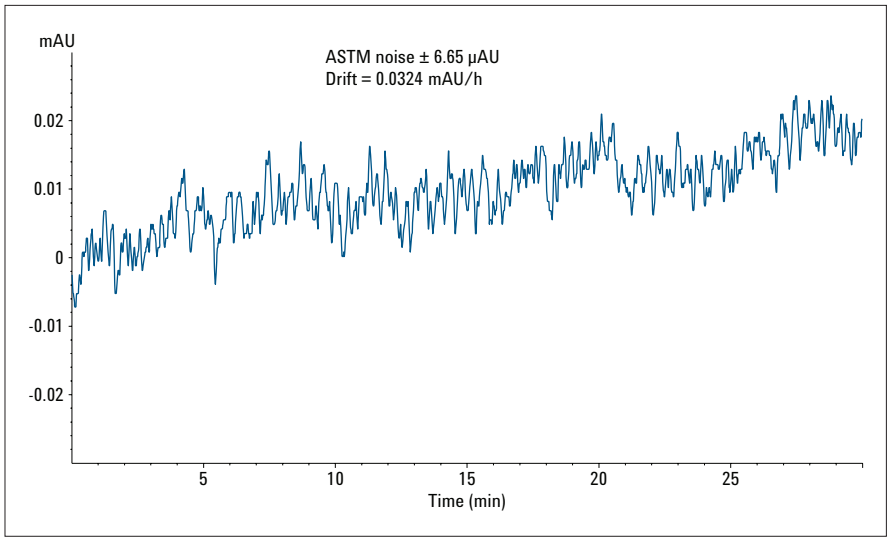
Chromatographic conditions	
Sample:	Agilent 1200 Series Rapid Resolution LC system checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Mobile phase:	A = Water B = Acetonitrile
Gradient:	30% B to 95% B in 1 minute
Flow rate:	3.4 mL/min
Stop time:	1.5 minutes
Post-time:	1.0 minutes
Injection volume:	1 µL
Column temperature:	40 °C
Detection:	245 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.0063 minutes (0.13 second response time, 40 Hz)

Figure 3
Precision for a gradient run at 590 bar with a RT precision < 0.080% RSD.

Detector Performance

Noise and Drift

Evaluation of baseline noise was performed according to guidelines of the American Society for Testing and Materials (ASTM) in addition to drift measurements of the 10-mm path length flow cell. ASTM noise and drift was evaluated using a restriction capillary instead of a column and water as the mobile phase. The DAD was set to 1.25 Hz (4 second response). The resulting ASTM noise was ± 6.65 µAU and the drift was 0.0324 mAU.

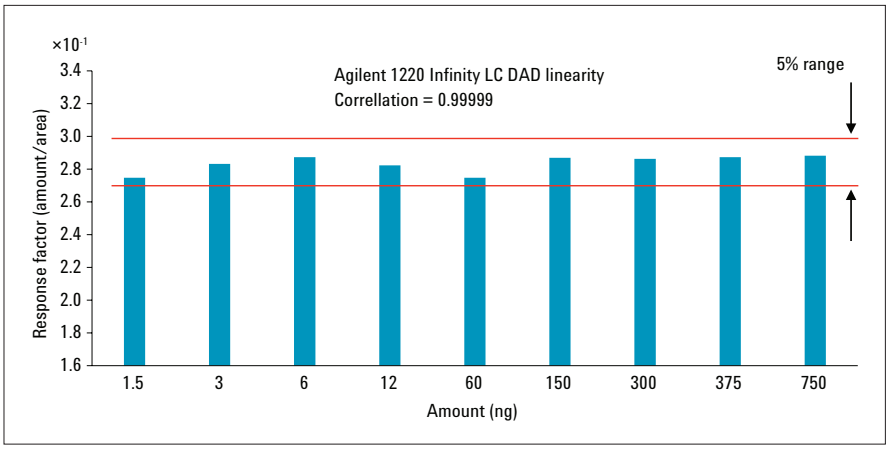


Chromatographic conditions	
Column:	Restriction capillary, backpressure 48 bar
Mobile phase:	Water, isocratic
Flow rate:	1.0 mL/min
Stop time:	30 minutes
Column temperature:	36 °C
Detection:	254 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.20 minutes (4.0 second response time, 1.25 Hz)

Figure 4
Noise and drift of the DAD.

Linearity for Different Caffeine Concentrations

Certified caffeine standards from 1.5 to 750 ng of injected amount were used to test detection linearity. Excellent linearity was obtained for this concentration range. The coefficient of correlation was 0.99999. The response factors were all within the ±5% error range from 1.5 to 750 ng, see Figure 5.

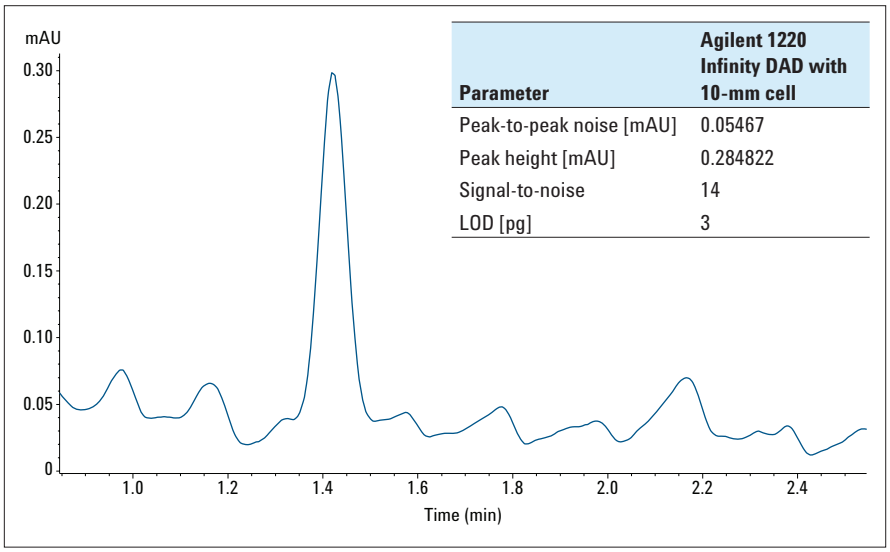


Chromatographic conditions	
Sample:	Certified caffeine standards
Column:	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-302)
Mobile phase:	A = Water B = Acetonitrile
Isocratic:	90/10 A/B
Flow rate:	0.8 mL/min
Injection volume:	3 µL
Stop time:	1.5 minutes
Column temperature:	30 °C
Detection:	273 nm/10 nm, Ref.: 360 nm/80 nm
Flow cell:	10 mm
Peak width:	> 0.013 minutes (0.25 second response time, 20 Hz)

Figure 5
Linearity using certified caffeine standards as sample compound.

Limit of Detection for Anthracene

The limit of detection (LOD) for anthracene was evaluated using the DAD at 2.5 Hz. The injected concentration was as low as 5 pg/µL, see Figure 6. The LOD for a signal-to-noise ratio of 3 was 3 pg.



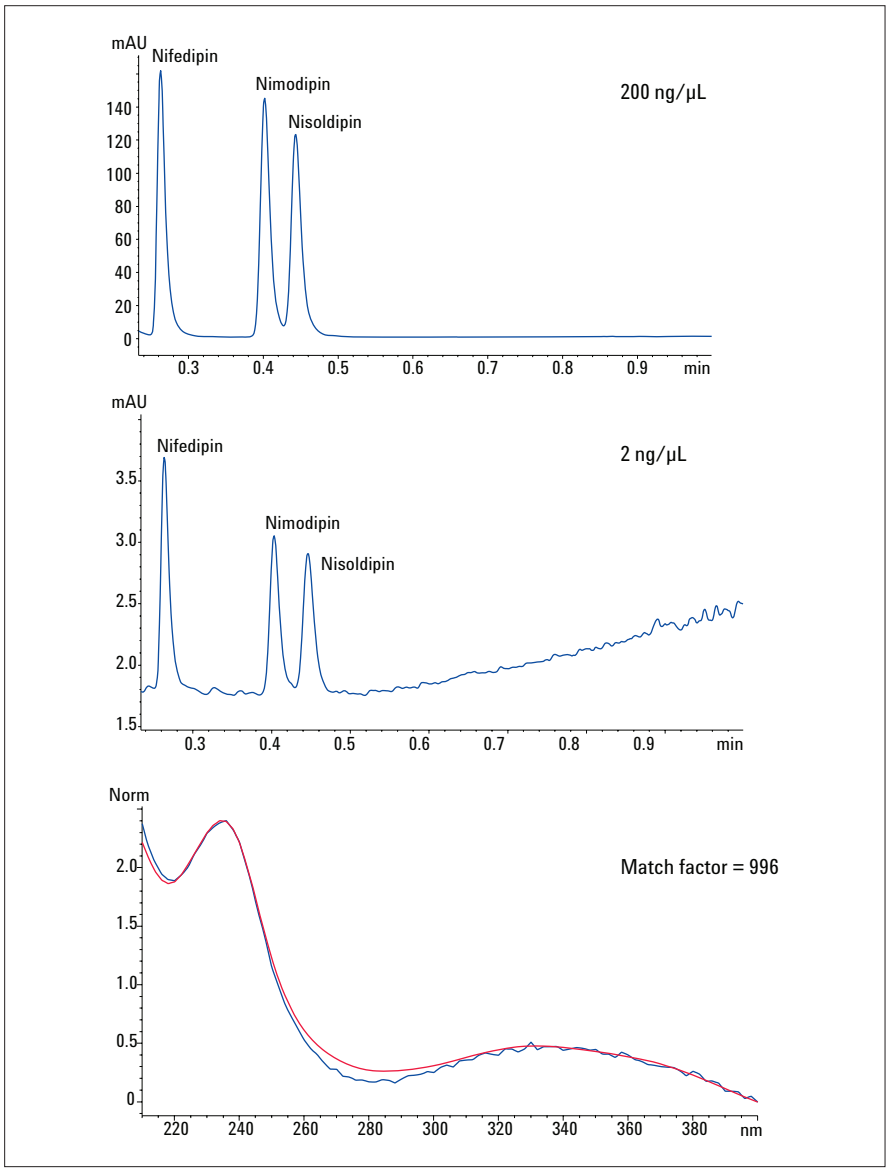
Parameter	Agilent 1220 Infinity DAD with 10-mm cell
Peak-to-peak noise [mAU]	0.05467
Peak height [mAU]	0.284822
Signal-to-noise	14
LOD [pg]	3

Chromatographic conditions	
Sample:	Anthracene, 5 pg in 1 µL
Column:	Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)
Mobile phase:	A = Water B = Acetonitrile
Isocratic:	35/65 A/B
Flow rate:	0.5 mL/min
Stop time:	3.0 minutes
Column temperature:	36 °C
Detection:	251 nm/4 nm, Ref.: 450 nm/80 nm, 8 mm slit width
Flow cell:	10 mm
Peak width:	> 0.10 minutes (2.0 second response time, 2.5 Hz)

Figure 6
Limit of detection for anthracene.

Spectral Conformation of Trace-level Compounds Using Ultrafast Chromatographic Conditions

A library search was performed for the measured trace-level spectrum. Match factors were calculated and tabulated in the library research table. Highest spectral match was achieved for nifedipin with a match factor of 996. The spectral library analysis confirmed the compound identification based on chromatographic retention. This positive spectral confirmation significantly enhances confidence in qualitative analytical results, see Figure 7.



Chromatographic conditions	
Sample:	Nifedipin, nimodipin, and nisoldipin each 200 ng/µL and 2 ng/µL
Column:	Agilent ZORBAX RRHT SB C18, 4.6 × 50 mm, 1.8 µm (p/n 827975-902)
Mobile phase:	A = Water + 0.05% TFA B = Acetonitrile + 0.045% TFA
Gradient:	65% B to 70% B in 0.85 minutes
Flow rate:	3.0 mL/min
Injection volume:	1 µL, needle cleaning with methanol
Column temperature:	36 °C
Detection:	254 nm/4 nm, Ref.: 450 nm/80 nm, 8 mm slit width, all spectra
Stop time:	1 minute
Post time:	1 minute

Figure 7
Analysis of nifedipin, nimodipin, and nisoldipin for spectral and purity evaluation.

Peak Purity Analysis of Trace-level Compounds Using Ultrafast Chromatographic Conditions

The 1220 Infinity Gradient LC System with DAD enables peak purity analysis under ultrafast LC conditions, even for trace-level compounds. The spectral analysis confirmed that the nifedipin peak, as identified by chromatographic retention, was pure. This positive purity confirmation significantly increases confidence in quantitative chromatographic results, see Figure 8.

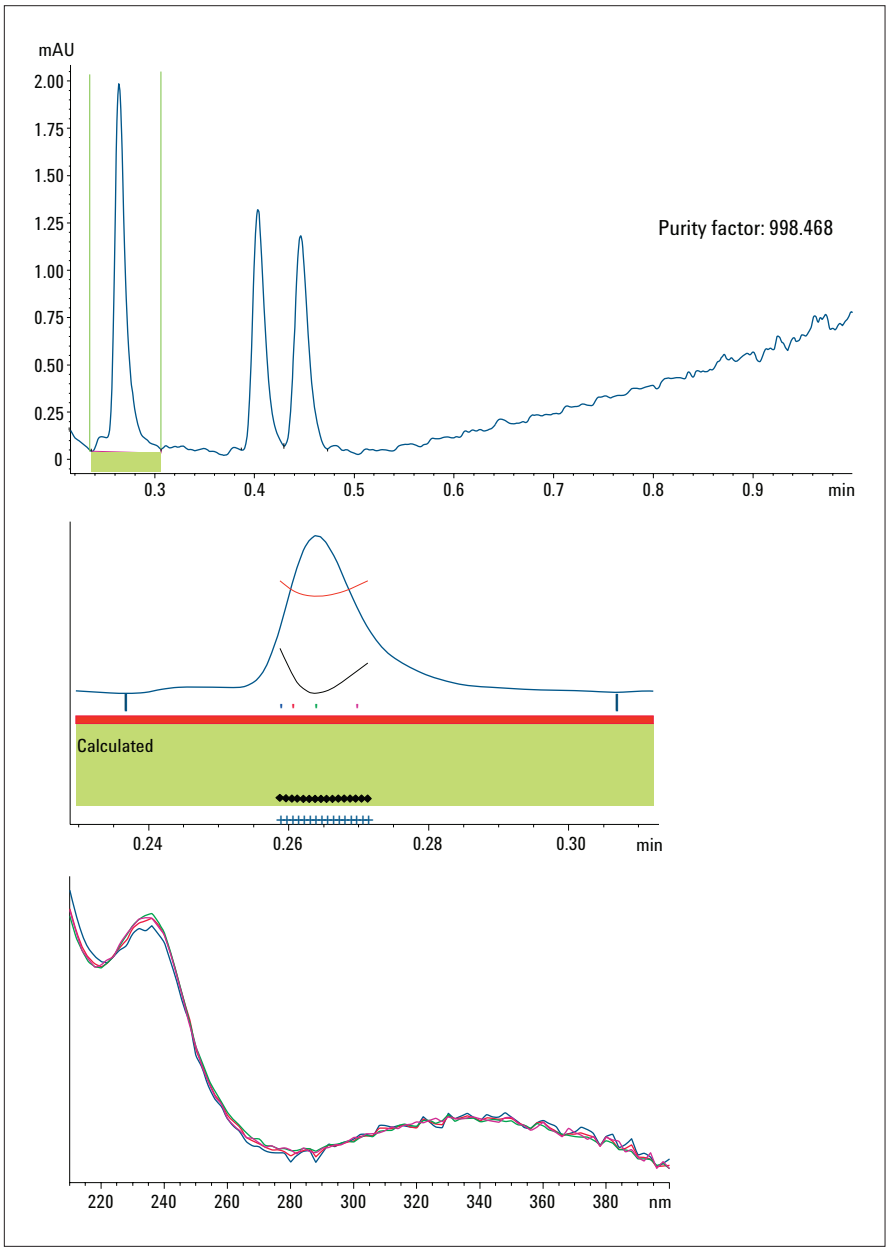


Figure 8
Analysis of nifedipin, nimodipin, and nisoldipin for spectral and purity evaluation.

Performance After Vibration Tests

To prove the robustness and ruggedness of the 1220 Infinity Gradient LC System with a DAD as a mobile solution, the system was subject to vibration tests on a moving tray. After the vibration tests, pump and autosampler performance was repeated as well as noise and drift measurement of the DAD.

Table 1 shows the results for the RSD values with respect to retention time and area before and after the vibration test. The values are all in a similar range before and after the test, resulting in the conclusion that the vibration did not affect pump and autosampler performance referring to RSD of retention times and areas.

Table 2 shows the results of the detector performance tests including the noise and drift measurements before and after the vibration tests. No major differences were found between the analysis before and after vibration.

Conclusions

The Agilent 1220 Infinity Gradient LC system is now equipped with a DAD for multiwavelength detection and spectra analysis. This Technical Overview shows that the performance of the 1220 Infinity LC system with a DAD meets the requirements of modern analytical liquid chromatography. The 1220 Infinity Gradient LC system with a DAD is a mobile solution for onsite measurement, which is proved by the reproducibility of very good RSD values for retention time and area before and after vibration tests. In addition, noise and drift measurements gave very similar results before and after vibration, showing that the 1220 Infinity Gradient LC system is a robust and rugged system for onsite measurement. The detailed performance analysis of the DAD revealed high linearity and sensitivity with optional spectral confirmation analysis.

	% RSD RT		% RSD area	
Experiment	**Test**	**After test**	**Before test**	**After test**
Long gradient run	< 0.063	< 0.069	< 1.6	< 1.0
Short gradient run	< 0.08	< 0.125	< 0.9	< 0.6
Isocratic run	< 0.014	< 0.011	< 0.055	< 0.045

Table 1
RSD of RT and area before and after vibration tests.

Experiment	Result
Noise	< 10 µAU (before and after vibration test)
Drift	< 0.04 (before and after vibration test)
Detector linearity	0.99999
LOD anthracene	3 pg

Table 2
Detector performance.

www.agilent.com/chem/1220

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