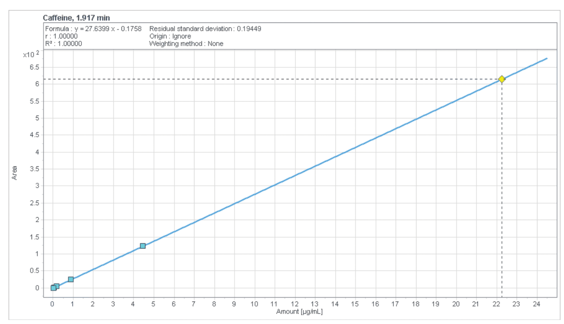
Technical Overview

Let Your Autosampler Do Your Pipetting

Using the injector program of the Agilent 1260 Infinity II Vialsampler and Multisampler for preparation of calibration solutions



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Abstract

The injector program available with Agilent autosamplers offers the possibility to automate liquid handling workflows such as the preparation of calibration solutions. The automation of liquid handling steps saves time and cost generated by manual work. This technical overview describes how the injector program in combination with the Agilent 1260 Infinity II Vialsampler or the Agilent 1260 Infinity II Multisampler can be used for preparation of calibration solutions and standard dilutions for estimation of detection and quantification limits (LOD and LOQ). Excellent linearity is obtained for calibrations using calibration solutions prepared by the autosamplers and, unlike using manually prepared calibration solutions, results are independent of the skill of the operator.

Introduction

Liquid handling workflows, such as the preparation of calibration solutions for quantification, are labor and time intensive, when performed manually. Furthermore, the quality of the results obtained using manually prepared calibration solutions are highly dependent on the skills of the operator.

Agilent autosamplers, such as the 1260 Infinity II Vialsampler and the 1260 Infinity II Multisampler, offer the possibility to use an injector program with the ability to define a series of operations that the autosampler carries out sequentially. The injector program can be used to employ the autosampler for preparation of calibration solutions, which avoids manual liquid handling steps and thereby saves time and cost generated by manual work. Furthermore, results obtained using calibration solutions prepared by the autosampler do not depend on the skills of the operator.

This technical overview describes how the injector program in combination with the Agilent 1260 Infinity II Prime LC System with 1260 Infinity II Vialsampler or 1260 Infinity II Multisampler can be used for preparation of calibration solutions. Calibration solutions are prepared by serial dilution, exemplified using caffeine as sample. The results obtained using calibration solutions prepared by the autosampler are compared to results obtained from manual preparation of calibration solutions. To enable comparison, a control sample is analyzed and evaluated with both calibrations. Serial dilution performed by the autosampler can also be employed for estimation of LOD and LOQ. The analysis of the serially diluted standards allows the determination of the concentration closest to the LOD, defined based on the signal-to-noise ratio (S/N).

Experimental

Equipment

The Agilent 1260 Infinity II Prime LC System comprised the following modules:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- *Either:* Agilent 1260 Infinity II Vialsampler (G7129C) with 100 µL analytical head and 100 µL loop (default setup) with sample thermostat (option #101)
- *Or:* Agilent 1260 Infinity II Multisampler (G7167A) with 100 µL analytical head and 100 µL loop (default setup) or with 900 µL analytical head (option #163) and 900 µL loop (option #156) with sample thermostat (option #101)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A)
- Agilent 1260 Infinity II Diode Array Detector HS (G7117C) with InfinityLab Max-Light cartridge cell, 10 mm (G4212-60008)

Software

Agilent OpenLab CDS Version 2.3.

Columns

- Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm (p/n 699775-902)
- Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)

Chemicals

All solvents were LC grade. Acetonitrile was purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak, EMD Millipore, Billerica, MA, USA). Caffeine was purchased from Sigma-Aldrich (Steinheim, Germany).

Samples

Caffeine is used as sample to show the use of the injector program for preparation of calibration solutions. Serial five-fold dilutions are performed from a 100 µg/mL caffeine stock solution in water/acetonitrile (98/2; v/v). Seven dilutions of the stock solution are prepared, leading to calibration solutions containing 20, 4, 0.8, 0.16, 0.032, 0.006, and 0.001 µg/mL caffeine. Calibration is performed in the range of 0.006 to 20 µg/mL. The 0.006 µg/mL calibration solution is further diluted for estimation of the LOD based on the S/N.

The results obtained using the calibration solutions prepared by the autosampler are compared to results obtained from manual preparation of calibration solutions. To enable comparison, a caffeine control sample with a concentration of approximately 9 µg/mL is analyzed and evaluated with both calibrations.

Sample preparation method (injector program)

Employing the 1260 Infinity II Vialsampler or the 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop, a serial five-fold dilution with a total volume of 100 µL is performed. Figure 1 shows the injector program used to obtain a five-fold dilution of the caffeine stock solution employing a 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop. Table 1 explains the individual steps performed.

The injector program shown in Figure 1 can analogously be used with the 1260 Infinity II Vialsampler with 100 µL analytical head and 100 µL loop.

Function	Parameter
Eject	Eject maximum volume to location "D1F-F1" with default speed using default offset
Draw	Draw 80.00 µL from location "D1F-E1" with default speed using default offset
Draw	Draw 20.00 µL from location "D1F-D1" with default speed using default offset
Wash	Wash needle as defined in method
Eject	Eject maximum volume to location "D1F-D2" with default speed using default offset
Repeat	Repeat 3 time(s)
Draw	Draw maximum volume from air with default speed
Eject	Eject 80.00 µL to location "D1F-D2" with default speed using default offset
Eject	Eject maximum volume to location "D1F-F1" with default speed using default offset
End Repeat	End Repeat
Wash	Wash needle as defined in method
Valve	Switch valve to "Mainpass"
Wait	Wait 0.6 min
Valve	Switch valve to "Bypass"

Figure 1. Injector program for a five-fold dilution performed using the Agilent 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop.

Table 1. Explanation of injector program for a five-fold dilution performed using the Agilent 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop.

Step		Explanation	Comment
Function	Parameter		
Eject	Eject maximum volume to location “D1F-F1” with default speed using default offset	The metering device is moved to its initial position.	Position “D1F-F1” holds an empty 2 mL screw top vial (p/n 5182-0715) as waste vial. The vial is capped without a septum to avoid carryover.
Draw	Draw 80 µL from location “D1F-E1” with default speed using default offset	80 µL of the diluent are drawn into the loop.	Position “D1F-E1” holds the diluent in a 2 mL screw top vial (p/n 5182-0715). A cap with a preslit septum (p/n 5185-5824) is used.
Draw	Draw 20 µL from location “D1F-D1” with default speed using default offset	20 µL of the caffeine stock solution are drawn into the loop.	Position “D1F-D1” holds 100 µL of the caffeine stock solution in a 2 mL screw top vial (p/n 5182-0715) with a 400 µL glass insert (p/n 5181-3377). A cap with a preslit septum (p/n 5185-5824) is used.
Wash	Wash needle as defined in method	The needle is washed to avoid carryover.	
Eject	Eject maximum volume to location “D1F-D2” with default speed using default offset	The diluent and caffeine stock solution are ejected to an empty vial.	Position “D1F-D2” holds an empty 2 mL screw top vial (p/n 5182-0715) with a 400 µL glass insert (p/n 5181-3377). A cap with a preslit septum (p/n 5185-5824) is used.
Repeat	Repeat three times	The caffeine stock solution is mixed with the diluent. Mixing is performed with air.	
Draw	Draw maximum volume from air with default speed		
Eject	Eject 80 µL to location “D1F-D2” with default speed using default offset		
Eject	Eject maximum volume to location “D1F-F1” with default speed using default offset		
End Repeat	End repeat		
Wash	Wash needle as defined in method	The needle is washed to avoid carryover.	
Valve	Switch valve to mainpass	The needle and loop are flushed by the pump flow before the next dilution is performed.	
Wait	Wait 0.6 minutes		
Valve	Switch valve to bypass		

Note that the draw and eject speed "default" use the values from the acquisition method. In the same way, the needle height offset "default" also uses the settings from the acquisition method. The acquisition method used for caffeine analysis employing the 1260 Infinity II Vialsampler or the 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop is shown in Table 2.

The injector program shown in Figure 1 is repeated seven times within one sample preparation method to obtain seven serial five-fold dilutions of the caffeine stock solution. In the repetitions of the injector program, the stock and destination vial locations are consecutively changed. Figure 2 shows the sequence used for preparation and analysis of the caffeine calibration standards as well as the control sample. The seven dilutions are performed within one injector program and the prepared calibration standards are subsequently analyzed.

Table 2. Acquisition method for caffeine analysis using the Agilent 1260 Infinity II Vialsampler or the Agilent 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 µm
Solvent	A) Water B) Acetonitrile
Gradient	0.00 minutes – 5% B 3.00 minutes – 50% B 3.10 minutes – 90% B Stoptime: 4 minutes Post-time: 3 minutes
Flow rate	0.500 mL/min
Temperature	40 °C
Detection	273 nm/4 nm, reference 360 nm/100 nm 20 Hz
Injection	**Agilent 1260 Infinity II Vialsampler with 100 µL analytical head and 100 µL loop**
	Injection volume: 5 µL Sampling speeds: draw speed: 200 µL/min; eject speed: 400 µL/min (default values) Needle height position: offset 0.0 mm Sample temperature: 8 °C Needle wash: 3 seconds in water/acetonitrile (50/50)
	Agilent 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop
	Injection volume: 5 µL Sampling speeds: draw speed: 100 µL/min; eject speed: 400 µL/min (default values) Needle height position: use vial bottom sensing, offset –1.0 mm Sample temperature: 8 °C Needle wash: 3 seconds in water/acetonitrile (50/50)

		Action	Vial	Sample type	Level	Acq. method	Volume	Sample name	Data file	Sample prep method
1	☑	Inject	D1F-C1	Blank		Caffeine_gradient.amx	Use Method	Blank	<S> <001>	
2	☑	Inject	D1F-C1	Sample		Caffeine_gradient.amx	Use Method	Injector Program	<S> <002>	Dilution_Sampler_MLS_Final.smx
3	☑	Inject	D1F-C1	Blank		Caffeine_gradient.amx	Use Method	Blank	<S> <003>	
4	☑	Inject	D1F-D8	Sample		Caffeine_gradient.amx	Use Method	Caffeine 0.001	<S> <004>	
5	☑	Inject	D1F-D7	Cal. Std.	1	Caffeine_gradient.amx	Use Method	Caffeine 0.007	<S> <005>	
6	☑	Inject	D1F-D6	Cal. Std.	2	Caffeine_gradient.amx	Use Method	Caffeine 0.036	<S> <006>	
7	☑	Inject	D1F-D5	Cal. Std.	3	Caffeine_gradient.amx	Use Method	Caffeine 0.18	<S> <007>	
8	☑	Inject	D1F-D4	Cal. Std.	4	Caffeine_gradient.amx	Use Method	Caffeine 0.89	<S> <008>	
9	☑	Inject	D1F-D3	Cal. Std.	5	Caffeine_gradient.amx	Use Method	Caffeine 4.45	<S> <009>	
10	☑	Inject	D1F-D2	Cal. Std.	6	Caffeine_gradient.amx	Use Method	Caffeine 22.26	<S> <010>	
11	☑	Inject	D1F-D1	Sample		Caffeine_gradient.amx	Use Method	Caffeine 111.3	<S> <011>	
12	☑	Inject	D1F-C1	Blank		Caffeine_gradient.amx	Use Method	Blank	<S> <012>	
13	☑	Inject	D1F-B1	Sample		Caffeine_gradient.amx	Use Method	Caffeine Control	<S> <013>	
14	☑	Inject	D1F-B1	Sample		Caffeine_gradient.amx	Use Method	Caffeine Control	<S> <014>	
15	☑	Inject	D1F-B1	Sample		Caffeine_gradient.amx	Use Method	Caffeine Control	<S> <015>	
16	☑	Inject	D1F-C1	Blank		Caffeine_gradient.amx	Use Method	Blank	<S> <016>	

Figure 2. Sequence used for preparation and analysis of the caffeine calibration solutions as well as the control sample employing the Agilent 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop.

Employing the 1260 Infinity II Multisampler with 900 µL analytical head and 900 µL loop, a serial five-fold dilution with an increased total volume of 900 µL can be performed. The injector program used to obtain a five-fold dilution with a total volume of 900 µL from the caffeine stock solution is shown in Figure 3. The individual steps performed are the same as explained in Table 1, but higher volumes are used, and the vials are used without inserts.

Table 3 shows the acquisition method used for caffeine analysis employing the 1260 Infinity II Multisampler with 900 µL analytical head and 900 µL loop. A higher flow rate is chosen to enable faster flushing of the 900 µL loop in between the dilutions. The column and injection volume are chosen accordingly.

Function	Parameter
Eject	Eject maximum volume to location "D1F-F1" with default speed using default offset
Draw	Draw 720.00 µL from location "D1F-E1" with default speed using default offset
Draw	Draw 180.00 µL from location "D1F-D1" with default speed using default offset
Wash	Wash needle as defined in method
Eject	Eject maximum volume to location "D1F-D2" with default speed using default offset
Repeat	Repeat 3 time(s)
Draw	Draw maximum volume from air with default speed
Eject	Eject 720.00 µL to location "D1F-D2" with default speed using default offset
Eject	Eject maximum volume to location "D1F-F1" with default speed using default offset
End Repeat	End Repeat
Wash	Wash needle as defined in method
Valve	Switch valve to "Mainpass"
Wait	Wait 1.13 min
Valve	Switch valve to "Bypass"

Figure 3. Injector program for a five-fold dilution performed using the Agilent 1260 Infinity II Multisampler with 900 µL analytical head and 900 µL loop.

Table 3. Acquisition method for caffeine analysis using the Agilent 1260 Infinity II Multisampler with 900 µL analytical head and 900 µL loop.

Parameter	Value
Column	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm
Solvent	A) Water B) Acetonitrile
Gradient	0.00 minutes – 5% B 3.00 minutes – 50% B 3.10 minutes – 90% B Stoptime: 5 minutes Post-time: 4 minutes
Flow rate	2.400 mL/min
Temperature	40 °C
Detection	273 nm/4 nm, reference 360 nm/100 nm 20 Hz
Injection	**Agilent 1260 Infinity II Multisampler with 900 µL analytical head and 900 µL loop**
	Injection volume: 24 µL Sampling speeds: draw speed: 450 µL/min; eject speed: 1500 µL/min (speed is lower than the default values) Needle height position: Use vial bottom sensing, Offset: –1.0 mm Sample temperature: 8 °C Needle wash: 3 seconds in water/acetonitrile (50/50)

Results and discussion

The use of the injector program for preparation of calibration solutions is shown using caffeine as sample. The results obtained from the analysis of calibration solutions prepared by the autosampler are compared to results obtained from manual preparation of calibration solutions. This comparison is achieved employing a caffeine control sample, which is analyzed and evaluated with both calibrations.

As an example, Figure 4 shows the analysis of a 20 µg/mL caffeine calibration solution prepared using the 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop.

The calibration curve obtained from the analysis of the caffeine calibration solutions in the concentration range of 0.006 to 20 µg/mL prepared using the 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop shows excellent linearity (see Figure 5). The quantifications of the caffeine control sample employing the calibration obtained using the calibration solutions prepared by the 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop and the manually prepared calibration solutions show a very low deviation of 1.08%. Assuming correct operation and use of the pipettes during manual preparation of the calibration solutions, this low deviation shows that the dilutions prepared by the 1260 Infinity II Multisampler with 100 µL analytical head

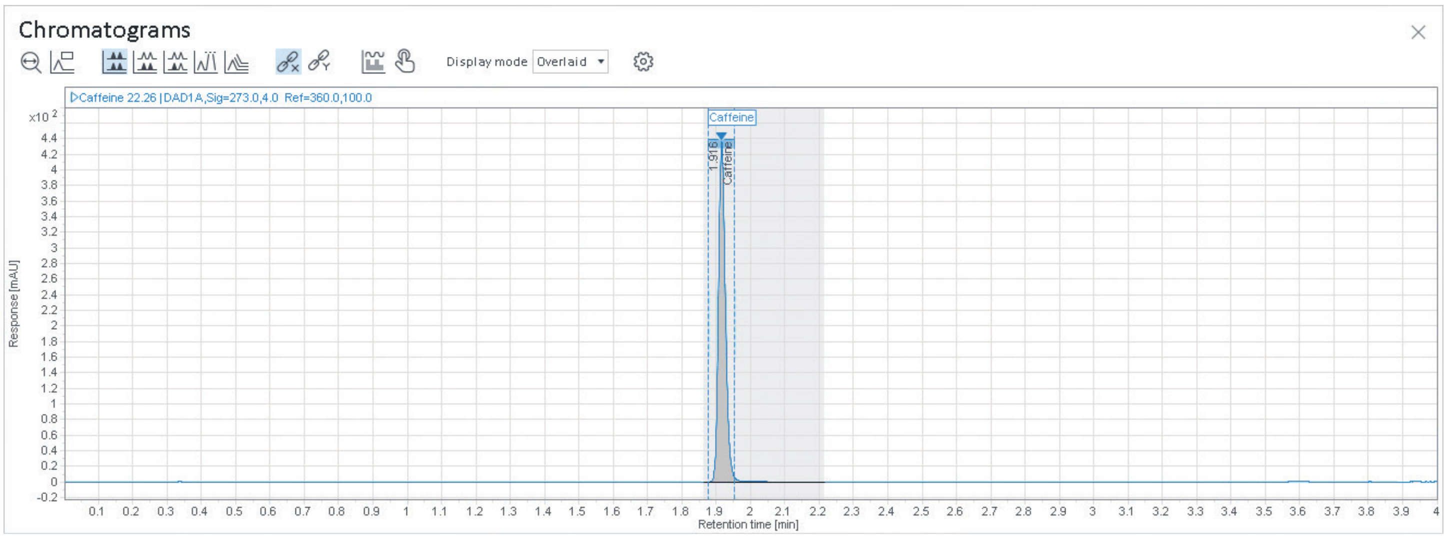

Figure 4. Analysis of a 20 µg/mL caffeine calibration solution prepared using the Agilent 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop.

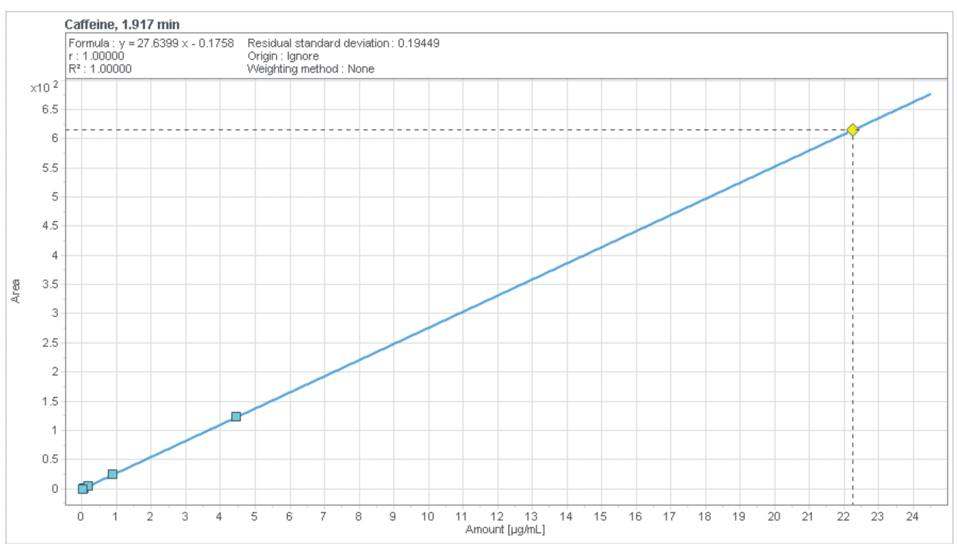

Figure 5. Calibration curve obtained from analysis of 0.006 to 20 µg/mL caffeine calibration solution prepared using the Agilent 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop.

and 100 µL loop are correct. Incorrect pipetting during manual preparation of calibration solutions could also lead to false calibration results from manually prepared calibration solutions and thereby a higher deviation.

Table 4 summarizes the caffeine calibrations obtained using calibration solutions that were manually prepared or prepared by the 1260 Infinity II Vialsampler or the 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop or the 1260 Infinity II Multisampler with 900 µL analytical head and 900 µL loop. Excellent linearity was observed especially using the calibration solutions that were prepared by the autosamplers. In addition, the calibration functions are very comparable. Consequently, excellent agreement of the quantifications of the caffeine control sample between using the manually and autosampler-prepared calibration solutions is obtained.

Serial dilution performed by the autosampler can additionally be employed for estimation of LOD and LOQ. The LOD of an analytical procedure is defined as the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. For estimation of the LOD based on S/N, an S/N between 3:1 or 2:1 is generally considered acceptable.[1] The analysis of the serially diluted caffeine solutions allows the determination of the concentration closest to the LOD based on the S/N of the caffeine peak.

Figure 6 shows the analysis of a 0.001 µg/mL caffeine solution prepared by serial five-fold dilution from a 100 µg/mL caffeine stock solution employing the 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop. The inset of the chromatogram shows the properties of the caffeine peak, which are displayed in the injection results. The caffeine peak possesses an S/N of 2.5, which means that the LOD for the determination of caffeine can be estimated as 0.001 µg/mL.

In this technical overview, the use of the injector program for preparation of calibration solutions is shown by employing the 1260 Infinity II Vialsampler and the 1260 Infinity II Multisampler. The injector program is also available with other Agilent autosamplers. For the preparation of calibration solutions, the installed analytical head and loop need to be considered.

Table 4. Caffeine calibrations employing manually and autosampler-prepared calibration solutions.

Autosampler	Calibration Means	Calibration Function	Correlation R^2	Deviation Control Sample (%)
Agilent 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop	Manual	y=27.85*x+0.53	0.99999	1.08
	Autosampler	y=27.64*x-0.18	1.00000	
Agilent 1260 Infinity II Vialsampler with 100 µL analytical head and 100 µL loop	Manual	y=27.76*x+0.43	0.99999	0.79
	Autosampler	y=27.65*x-0.73	0.99999	
Agilent 1260 Infinity II Multisampler with 900 µL analytical head and 900 µL loop	Manual	y=27.69*x+0.54	0.99999	0.72
	Autosampler	y=27.53*x+0.02	1.00000	

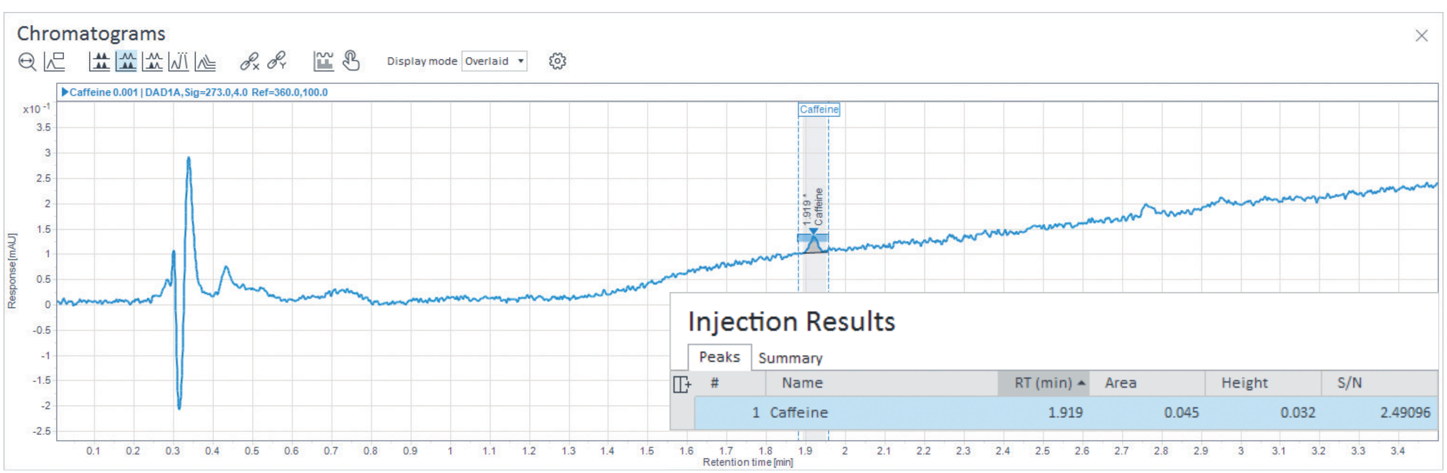

Figure 6. Estimation of LOD: analysis of a 0.001 µg/mL caffeine solution prepared by serial five-fold dilution from a 100 µg/mL caffeine stock solution employing the Agilent 1260 Infinity II Multisampler with 100 µL analytical head and 100 µL loop.

Conclusion

Agilent autosamplers, such as the 1260 Infinity II Vialsampler and the 1260 Infinity II Multisampler, offer the possibility to use the injector program for preparation of calibration solutions and standard dilutions for estimation of LOD and LOQ. This avoids manual liquid handling steps and thereby enables time and cost savings, and the results obtained are independent of the skill of the operator. Excellent linearity is obtained for calibrations using calibration solutions prepared by the autosamplers. Furthermore, excellent agreement of the quantifications of a control sample between using manually and autosampler-prepared calibration solutions is obtained.

Reference

1. International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, ICH harmonised tripartite guideline, VALIDATION OF ANALYTICAL PROCEDURES: TEXT AND METHODOLOGY Q2(R1), October 27, 1994. https://database.ich.org/sites/default/files/Q2_R1__Guideline.pdf (accessed December 2, 2019).

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