TEST CERTIFICATE

S-A-LE 405,1

DORSHORST 2
7217PH HARFSEN
NETHERLANDS

INSTRUMENT:	HPLC - VWD
ID NR.:	405,1
MODEL:	AGILENT SERIES 1100
CAL. DATE:	05 FEBRUARI 2019

HPLC-VWD

NR. 405,1: AGILENT SERIES 1100

GENERAL

In this report the performance of the Agilent Series 1100 HPLC system. Nr. 405,1 was investigated. The system consisted of a degasser, binary pump, autosampler, column oven compartment, a VWD detector and a PC with Chemstation software.

	Agilent Series		Serial	Number own system
Degasser	1100	G1379A	JP02200529	328,5
Binary pump	1100	G1312A	DE03007095	328,1
Autosampler	1100	G1313A	DE72003976	314
Column oven	1100	G1316A	DE53400976	216
VWD detector	1100	G1314A	JP92110038	405,1

The software package Chemstation contains several diagnosis test which can be used to check the operational performance of instrument modules (OQ tests). For each system the main diagnosis tests will be conducted. In case a test is failed, the cause will be investigated and the problem solved.

The diagnosis test phase will be finalized when all tests generate the qualification “passed”. Afterwards the system performance will be further tested in practice. For this a test application to Gallic Acid by HPLC-VWD has been developed and will be used (PQ tests).

SYSTEM DIAGNOSIS TESTS

The software package Chemstation contains several diagnosis test which can be used to check the operational performance of instrument modules and performance characteristics. The main diagnostic tests for the pump and detector tests were conducted. The results are presented in this chapter.

PRESSURE TEST

The Chemstation pressure test was conducted up to a final pressure of 390 bar using isopropanol as test solvent. The test was passed indicating (as shown down below in figure 1) that the pump and connecting tubing are in a good state.

	Limits	Measured	Result
Date: 1/29/2019; Time: 1:01:42 PM			
Slope of initial pressure ramp	> 300 bar/min	531 bar/min	Passed
Pressure value	> 385 bar	388 bar	Passed
Final pressure drop with flow 0	< 2 bar/min	1.9 bar/min	Passed

Figuur 1: Result of the pressure test

INTENSITY TEST

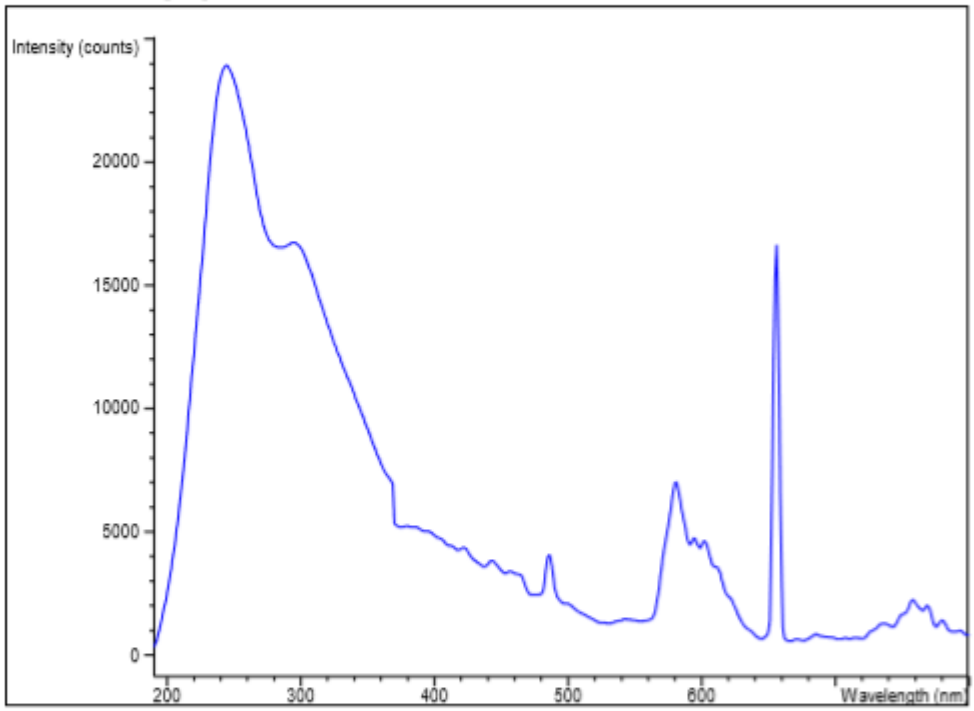
The intensity test checks the lamp intensities over the complete spectral range using MilliQ water as test solvent. The intensity test scans an intensity spectrum via the reference diode (200 - 1200 nm in 1 nm steps). The result of the scan is displayed in figure 2 down below.

Figure 2: Intensity spectrum

>>>>> VWD Intensity Test Report<<<<<

Instrument:	G1314A
Serial Number:	JP92110038
Operator:	Henk
Date:	9/25/2018
Time:	3:55:39 PM

VWD Intensity Spectrum



VWD Intensity Test Results

	Specification	Measured	Result
Accumulated lamp on time		96.31 h	
Highest intensity	> 10000 cts	23920 cts	Passed
Average intensity	> 5000 cts	5881 cts	Passed
Lowest intensity	> 200 cts	325 cts	Passed

SYSTEM PERFORMANCE (PQ TESTS)

The performance characteristics linearity, repeatability and detection limit were determined using a standard Gallic acid test method. The main details of the method, the solutions and the criteria are presented underneath:

METHOD

The following liquid chromatographic conditions were used:

- HPLC column	:	X select HSS T3, 3.5 µm particles, 4.6*100 mm column	
- Mobile phase A	:	95% MilliQ water/ 5% Methanol/ 0.1% TFA (v/v/v)	
- Mobile phase B	:	80% Methanol/ 20% MilliQ water/ 0.1% TFA (v/v/v)	
- Flow	:	1,0 ml/min	
- Injection volume	:	20 µl	
- Detector	:	VWD, wavelength 271 nm	
- Column temperature	:	Ambient	
- Quantification	:	External calibration, based on response factors	
- Gradient	:	T0 min	- 100% A - 0% B
		T7 min	- 100% A - 0% B
		T13 min	- 70% A - 30% B
		T13,5 min	- 0% A - 100% B
		T16,5 min	- 0% A - 100% B
		T16,6 min	- 100% A - 0% B

SOLUTIONS

Calibration standards were prepared using a stock solution containing 1000 ppm Gallic acid in methanol. The stock solution was diluted with a reconstitution solvent that contained 78% MilliQ water, 20% methanol and 2% formic acid (v/v/v) to reach the desired range of standard solutions. The calibration standards contained Gallic acid in the range of 0,36 to 25,0 mg/L.

CRITERIA

The acceptance criterion for the correlation coefficient is 0,999. The acceptance criterion for the relative standard deviation is 5,0%. There is no criterion for the detection limit (LOD), the LOD is defined as 3x noise level.

LINEARITY

The linearity was determined by measuring 5 calibration standard solutions in the range of 0,36 to 25,0 mg/L. The five point calibration curve of Gallic acid standard solutions in MilliQ water was recorded at 271 nm and is presented in Table 1 and Figure 5 below. The correlation coefficient of 0,9992 is within the specified acceptance criterion of 0,999.

Table 1: Results of the five point calibration curve

Concentration (mg/L)	Area (mAU)
0,36	21,5
1,5	91,6
3,0	182,8
6,0	366,3
25,0	1527,5

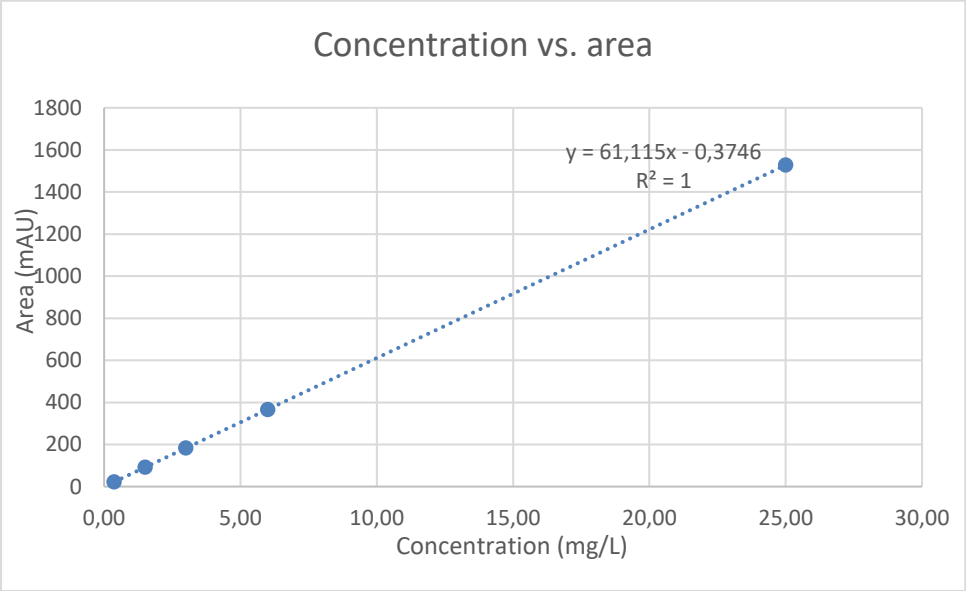


Figure 5: Graph of the five point calibration curve

REPEATABILITY

Repeatability was determined for the mid calibration standard with a concentration of 3,0 mg/L Gallic acid and is presented in Table 2 below. The relative standard deviation (0,17%) is within the acceptance criterion of 5,0% and therefore acceptable.

Table 2: Repeatability of the mid cal. standard

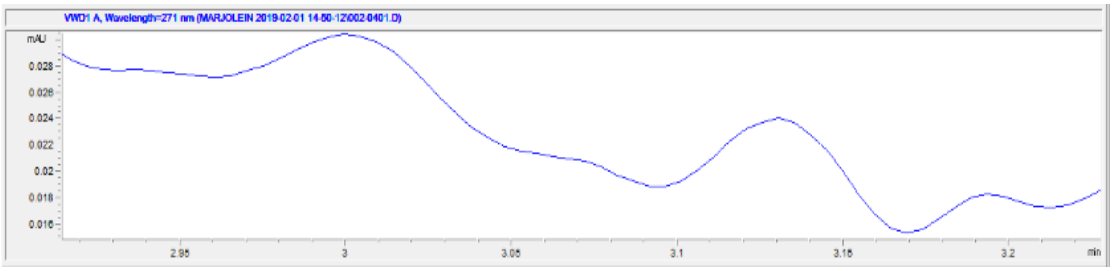
Number	Area (mAU)
3.1	420,2
3.2	419,5
3.3	420,1
3.4	419,7
3.5	420,2
3.6	420,1
Average	420.0
RSD (%)	0,07

DETECTION LIMIT

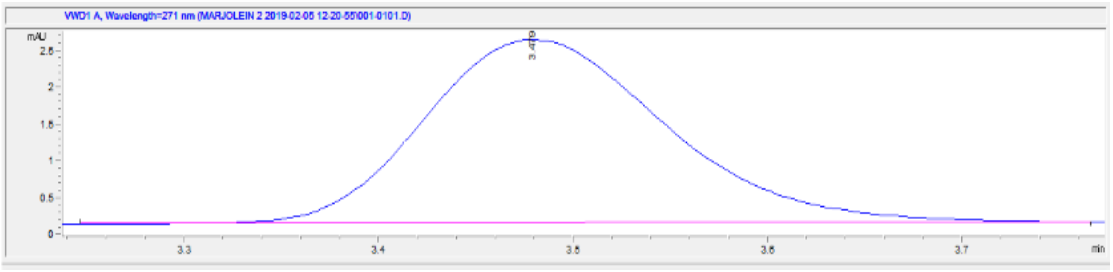
An example of the obtained chromatogram at the lowest calibration standard of 0,36 mg/l is presented below in Figure 6. From this chromatogram the detection limit (3x noise) was determined. The obtained detection limit was 0,036 mg/l for this substance.

Peak height: 2,5 mAU
Noise: 0,014
3x the noise 3x 0,014= 0,042 mAU
0,030/6,1*0,36= 0,006048

Figure 6: Obtained chromatogram lowest standard



Noise



Lowest standard

CONCLUSION

The system meets all the stated criteria, it is therefore in a good state and ready for use.

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