

TEST CERTIFICATE

S-A-LE 1337



DORSHORST 2
7217PH HARFSEN
NETHERLANDS

INSTRUMENT: HPLC – VWD
ID NR.: 1337
MODEL: AGILENT SERIES 1200
CAL. DATE: APRIL 16, 2021

HPLC - VWD

NR. 1337: AGILENT SERIES 1200

GENERAL

In this report the performance of the HPLC system, of combined Agilent Series 1200 instrument modules, was investigated. The system consisted of a degasser, quaternary pump, autosampler, column oven compartment, a variable wavelength detector (VWD) and a PC with Chemstation software.

Table 1: System specifications

Module	Model	Serial number
Degasser	G1322A	JP62359096
Quaternary pump	G1311A	DE62959563
Autosampler ALS	G1329A	DE64765140
Thermostatted column compartment (TCC)	G1316A	DE63064432
Variable wavelength detector (VWD)	G1314B	DE71361418

The Chemstation software package contains several diagnostic tests which can be used to check the operational performance of instrument modules (OQ tests). For some instrument modules, the main diagnosis tests were conducted. In case a test failed, the cause was investigated and the problem was solved. The diagnosis test phase was finalized when all tests generate the qualification “passed”.

Afterwards the system performance was further tested in practice. Therefore, a test application to Gallic Acid by HPLC-VWD has been developed and was used (PQ tests). We believe that these PQ tests are an essential element in establishing the quality of our systems.

DIAGNOSTIC TESTS (OQ TESTS)

The main diagnostic tests for the quaternary pump, column oven and VWD detector were conducted to check the operational performance of the individual instrument modules. The results are presented in this chapter. The operational characteristics of the autosampler and degasser were also checked. The autosampler was able to navigate to all positions and pick and drop vials. The degasser can achieve sufficient vacuum in the vacuum degasser, to show it is in a ready condition.

PRESSURE TEST

The pressure test was conducted for the quaternary pump up to a final pressure of 390 bar using isopropanol as test solvent. The test evaluates the pressure tightness of the system. The test was passed which indicates that the pump and connected tubing are in a good condition. The results are shown in Figure 1 and Table 2 below.

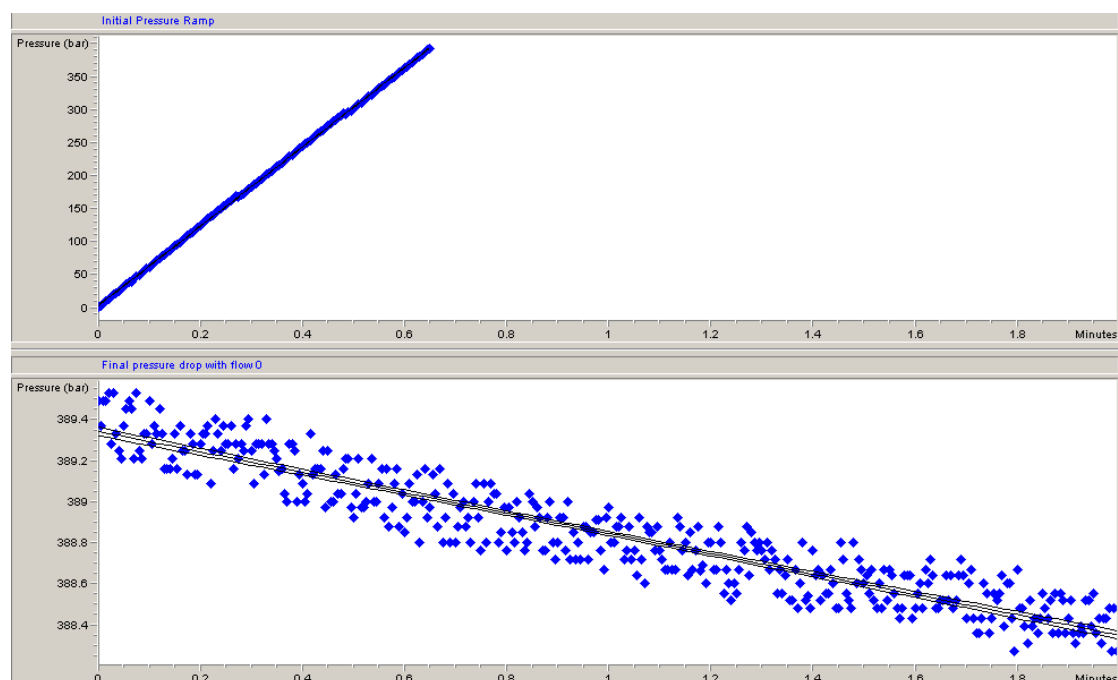


Figure 1: Pressure ramp and pressure drop of the quaternary pump.

Table 2: Results of the pressure test.

	Limits	Measured Result	
Date: 4/12/2021; Time: 5:03:11 AM			
Slope of initial pressure ramp	> 300 bar/min	601 bar/min	Passed
Pressure value	> 385 bar	389 bar	Passed
Final pressure drop with flow 0	< 2 bar/min	0.5 bar/min	Passed

THERMOSTAT TEST

The heating and cooling efficiency of the two Peltier elements of the thermostatted column compartment (TCC) was evaluated with the thermostat test. The results (Figure 2 and Table 3) show that it has passed the test and meets the requirements.

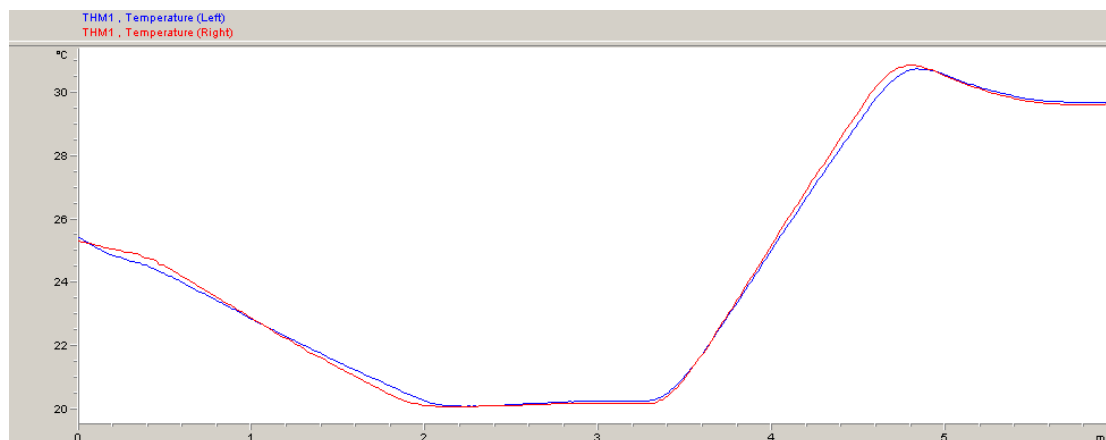


Figure 2: Cooling and heating rate for the left and right Peltier element of the column oven.

Table 3: Results of the thermostat test.

	Limits	Measured	Result
Date: 4/12/2021; Time: 5:13:26 At			
Cooling rate for left peltier element	>= 2 °C/min	2.8 °C/min	Passed
Heating rate for left peltier element	>= 3 °C/min	8.1 °C/min	Passed
Cooling rate for right peltier element	>= 2 °C/min	3.2 °C/min	Passed
Heating rate for right peltier element	>= 3 °C/min	8.5 °C/min	Passed

INTENSITY TEST

The performance of the deuterium lamp over the full VWD wavelength range (190 - 600 nm) was determined with the intensity test. The flow cell was also checked for dirty or contaminated windows. The detector has passed the test, the results of the scan are represented in Figure 3 and Table 4.

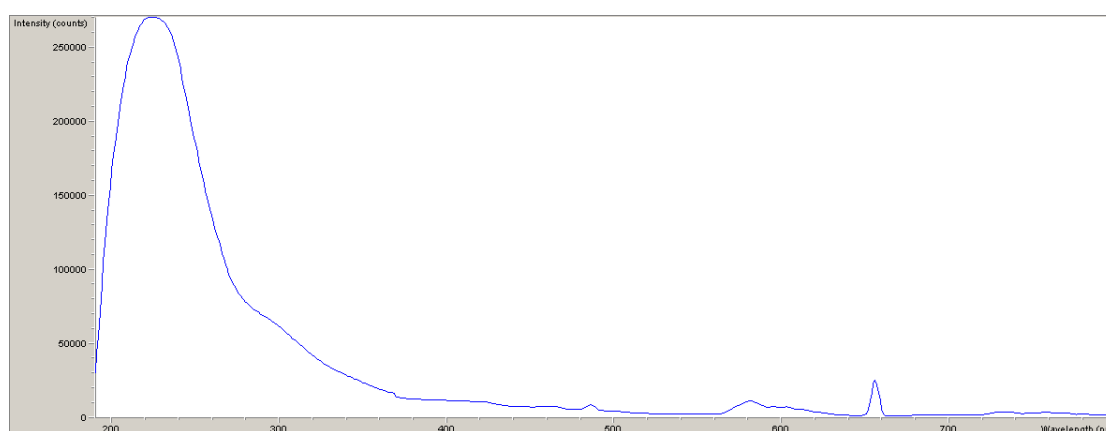


Figure 3: Intensity spectrum of the VWD detector.

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Table 4: Results of the intensity test.

	Limits	Measured	Result
Date: 4/12/2021; Time: 5:13:02 AM			
Accumulated lamp on time		803.06 h	
Highest intensity	> 10000 cts	270499 cts	Passed
Average intensity	> 5000 cts	36959 cts	Passed
Lowest intensity	> 200 cts	1020 cts	Passed

HOLIUM SPECTRUM

To verify the calibration of the detector, the holmium oxide test was performed. The wavelengths were evaluated against the three wavelength maxima of the built-in holmium oxide filter. The difference between the expected and measure maxima are displayed in Figure 4 and Table 5.

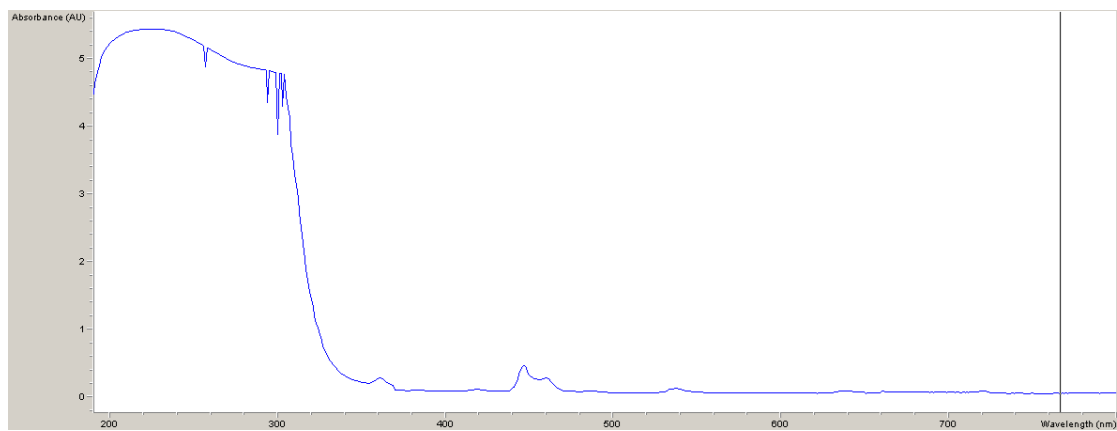


Figure 4: Holmium spectrum of the VWD detector.

Table 5: Results of the Holmium spectrum.

	Limits	Measured	Result
Date: 4/12/2021; Time: 5:18:24 AM			
Deviation from wavelength 1: 360.8 nm	-1..1 nm	0.0 nm	Passed
Deviation from wavelength 2: 418.5 nm	-1..1 nm	0.0 nm	Passed
Deviation from wavelength 3: 536.4 nm	-1..1 nm	0.1 nm	Passed

SYSTEM PERFORMANCE

The performance characteristics linearity, repeatability, limit of detection, carryover effect, injection volume and pressure ripple were determined using a standard Gallic acid test method. The main details of the method, the solutions and the criteria are presented below.

METHOD

The following liquid chromatographic conditions were applied:

- HPLC column : Confidential
- Mobile phase A : Confidential
- Mobile phase B : Confidential
- Flow : 0,75 ml/min
- Injection volume : 20 µl
- VWD detector : Confidential
- Column temperature : 40 °C
- Quantification : External calibration, based on peak areas/response factors
- Gradient :

T0 min	-	100%	A	-	0%	B
T7 min	-	100%	A	-	0%	B
T11 min	-	85%	A	-	15%	B
T11,5 min	-	5%	A	-	95%	B
T11,51 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 to reach the desired range of standard solutions. The calibration standards contained Gallic acid in the range of 0,36 to 25,0 ppm.

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 limit of detection (LoD), the LoD is defined as 3x noise level. The carryover effect is acceptable if it is less than 2%. The maximum acceptable deviation of the injection volume is 5%. The pressure ripple is acceptable when it deviates less than 1%.

LINEARITY

The linearity was determined by measuring 5 calibration standard solutions in the range of 0,36 to 25 ppm. The five-point calibration curve was recorded with a VWD detector. The results of the analysis are shown in Figure 5 and Table 6 below. The correlation coefficient of 1 comply with the specified acceptance criterion of $r > 0,999$.

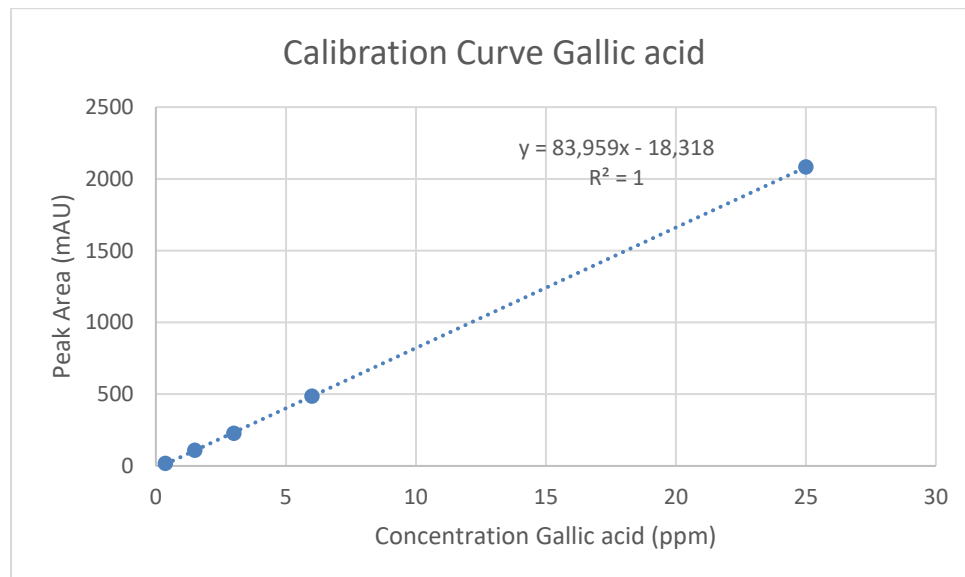


Figure 5: Graph of the five-point calibration curve with the VWD detector.

Table 6: Results of the five-point calibration curve.

Concentration (ppm)	Area (mAU)
0,36	17,1938
1,5	107,884
3	227,307
6	485,498
25	2081,31

REPEATABILITY

The repeatability was determined for the mid calibration standard with a concentration of 3 ppm gallic acid. The results are presented in Table 7. The relative standard deviation is within the acceptance criterion of 5,0% and it is therefore acceptable.

Table 7: Repeatability of the mid calibration standard.

Number	Area (mAU)
3.1	224,531
3.2	224,200
3.3	223,628
3.4	223,502
3.5	223,105
3.6	223,922
Average	223,81
STD	0,51
RSD	0,23 %

LIMIT OF DETECTION

The detection limit was derived from the peak height of lowest calibration standard and the height of the noise. The calculations and the obtained LoD of gallic acid are shown in the table below.

Table 8: Limit of detection for gallic acid.

	UV/VIS detector
Height noise (H1)	0,04 mAU
Peak height lowest calibration standard (H2)	2,20 mAU
Concentration lowest calibration standard (C)	0,36 ppm
LoD $((3*H1)/H2*C)$	0,0196 ppm

CARRYOVER EFFECT

The carryover effect of the method was determined based on the peak height of the sample when a blank solvent was injected directly after the highest calibration standard. The results (Table 9) show a carryover effect of less than 2% and passed.

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Table 9: Carryover effect of the method.

	UV/VIS detector
Peak height blank (H3)	0,009 mAU
Peak height highest calibration standard (H4)	203,10 mAU
Carryover effect (H3/H4*100%)	0,004 %

PRESSURE RIPPLE

The performance of the pump was assessed by determining the stability of the pressure in operation. The pressure ripple may have a maximum deviation of 1%. As shown in Figure 6 and Table 10, the pressure ripple for this method is 0,72%.

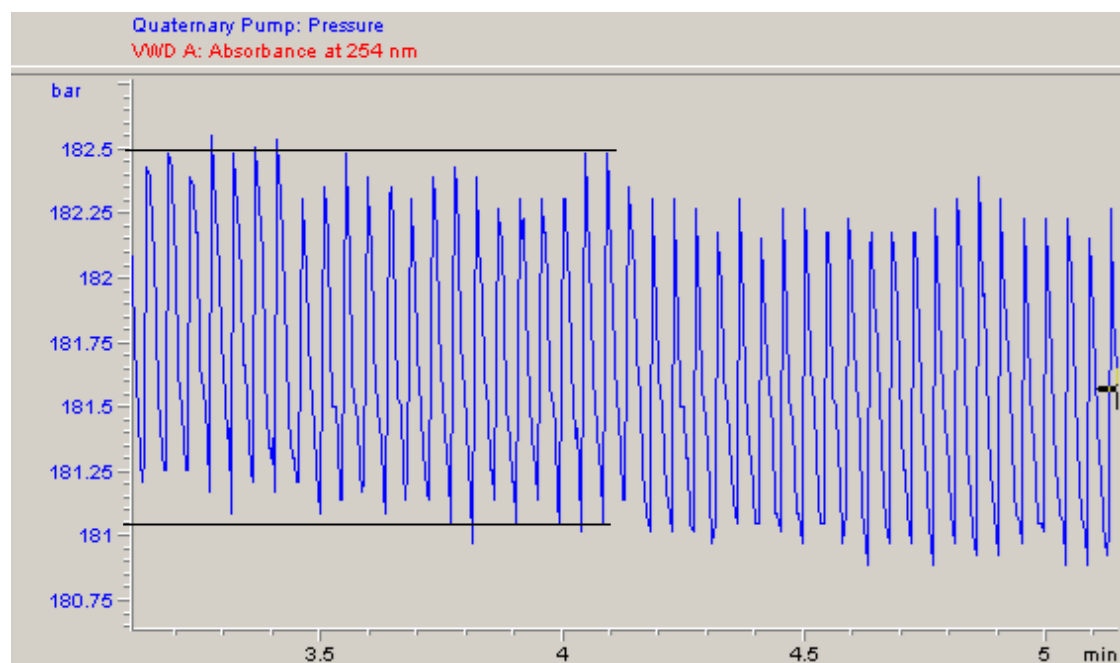


Figure 6: Display of pressure ripple during a run.

Table 10: Pressure ripple.

	Pressure
Highest pressure	182,48 bar
Lowest pressure	181,17 bar
Standard deviation (STD)	1,31 bar
Relative standard deviation (RSD)	0,72%

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CONCLUSION

The system passed the performed diagnostic tests and meets all the stated criteria for system performance. The system is therefore in a good state and ready to use.

AUTHORISATION: 16-04-2021



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