TEST CERTIFICATE

S-A-LE 1336

DORSHORST 2
7217PH HARFSEN
NETHERLANDS

INSTRUMENT:	HPLC – MWD
ID NR.:	1336
MODEL:	AGILENT SERIES 1200
CAL. DATE:	JANUARY 29, 2021

HPLC - MWD

NR. 1336: AGILENT SERIES 1200

GENERAL

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

Table 1: System specifications

Module	Model	Serial number
Degasser	G1322A	JP73066504
Quaternary pump	G1311A	DE62967411
Autosampler ALS	G1329A	DE64769369
Column oven compartment TCC	G1316A	DE63071223
Multiple wavelength detector (MWD)	G1365D	DE64255824

The Chemstation software package contains several diagnostic tests which can be used to check the operational performance of instrument modules (OQ tests). For some modules, the main diagnostic tests were conducted. In case a test failed, the cause was investigated and the problem was solved. The diagnostic 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-MWD has been developed and was used (PQ tests).

SYSTEM DIAGNOSIS TESTS

The main diagnostic tests for the quaternary pump, column oven and MWD 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.

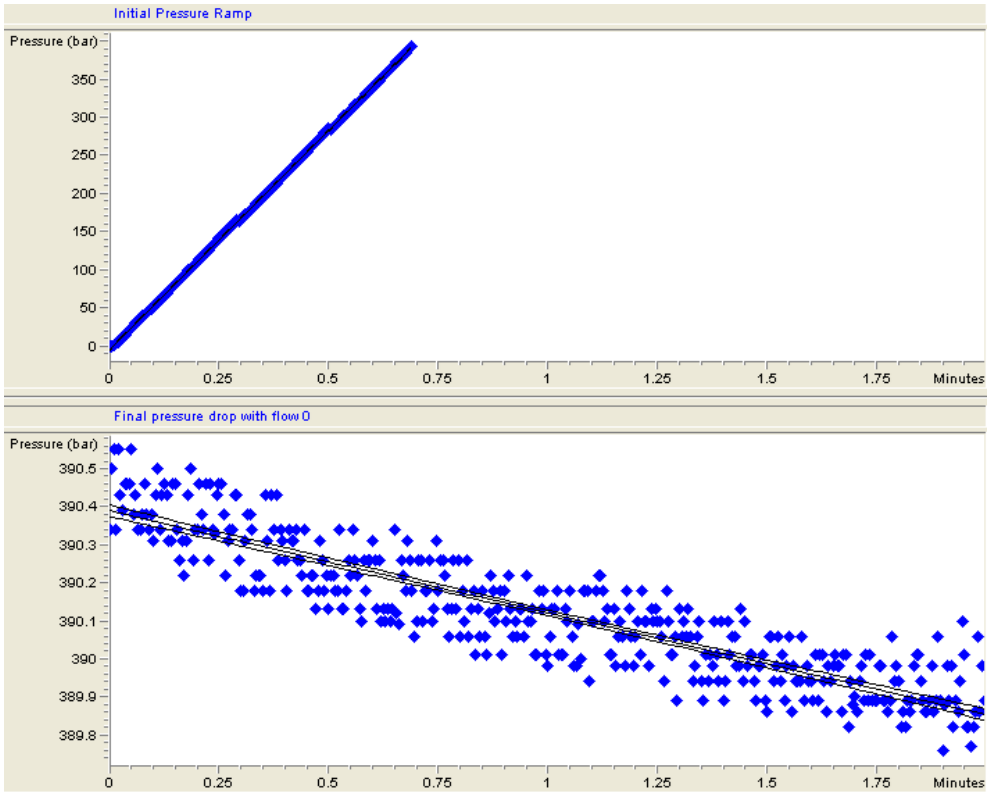

Figure 1: Pressure ramp and pressure drop of the quaternary pump.

Table 2: Results of the pressure test.

	Limits	Measured	Result
Date: 22/01/2021; Time: 02:02:28 PM			
Slope of initial pressure ramp	> 300 bar/min	575 bar/min	Passed
Pressure value	> 385 bar	390 bar	Passed
Final pressure drop with flow 0	< 2 bar/min	0.3 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.

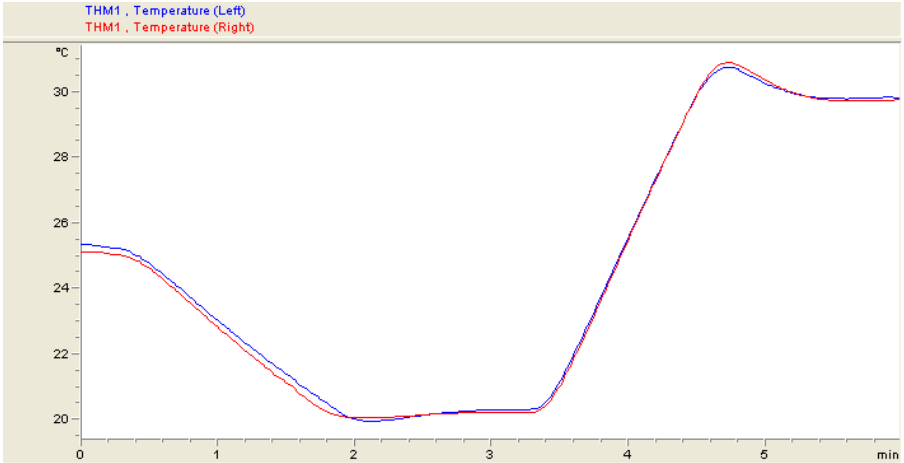

Figure 2: Cooling and heating rate for the left and right Peltier element of the column oven.

Table 3: Results of the thermostat test.

	Limits	Measured	Result
Date: 22/01/2021; Time: 02:12:36			
Cooling rate for left peltier element	>= 2 °C/min	3.4 °C/min	Passed
Heating rate for left peltier element	>= 3 °C/min	8.9 °C/min	Passed
Cooling rate for right peltier element	>= 2 °C/min	3.6 °C/min	Passed
Heating rate for right peltier element	>= 3 °C/min	9.0 °C/min	Passed

SELF TEST

The self test checks for among others the performance of the deuterium lamp over the full wavelength range (190 - 600 nm), calibration of the detector and the detector noise over a period of 20 minutes. The detector has passed all single tests, the results of the scan are represented in Table 4.

	Limits	Measured	Result
Filter test	0.005..0.5 AU	0.16 AU	Passed
Slit test	0.7..1.3	1.06	Passed
Dark current test	0..12000 cts	7729..7796 cts	Passed
Min. intensity (190nm - 220nm)	> 2000 cts	15087 cts	Passed
Min. intensity (221nm - 350nm)	> 5000 cts	21737 cts	Passed
Min. intensity (351nm - 500nm)	> 2000 cts	15235 cts	Passed
Min. intensity (501nm - 950nm)	> 4000 cts	12311 cts	Passed
Max. intensity (190nm - 350nm)	< 450000 cts	71184 cts	Passed
Max. intensity (700nm - 950nm)	< 300000 cts	56528 cts	Passed
Max. intensity (D2 alpha line)	< 1200000 cts	119587 cts	Passed
Wavelength at 486.0nm	485.5..486.5 nm	485.94 nm	Passed
Wavelength at 656.1nm	655.6..656.6 nm	655.99 nm	Passed
Holmium test	-1..1 nm	0.30 nm	Passed
Spectral flatness	< 0.002 AU	0.0000 AU	Passed
ASTM noise (20 min. at 254nm)	<= 0.02 mAU	0.0095 mAU	Passed

Table 4: Results of the MWD self-test.

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	:	Atlantis dC18, 3 µm particles, 3.0 x 150 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	:	0,75 ml/min					
-	Injection volume	:	20 µl					
-	MWD detector	:	Wavelength 271 nm					
-	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 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 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 of Gallic acid standard solutions was recorded with a MWD detector. The results of the analysis are shown in Figure 3 and Table 5 below. The correlation coefficient of 1 comply with the specified acceptance criterion of r > 0,999.

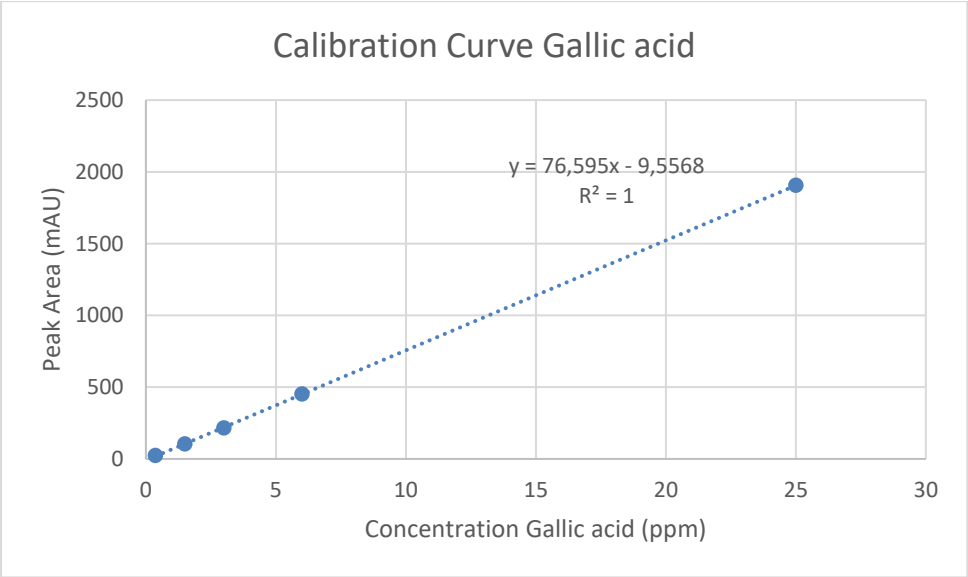

Figure 3: Graph of the five-point calibration curve with the MWD detector.

Table 5: Results of the five-point calibration curve.

Concentration (ppm)	Area (mAU)
0,36	22,5809
1,5	104,532
3	214,747
6	451,437
25	1905,62

REPEATABILITY

The repeatability was determined for the mid calibration standard with a concentration of 3 ppm gallic acid. The results are presented in Table 6. The relative standard deviation is within the acceptance criterion of 5,0% and therefore acceptable.

Table 6: Repeatability of the mid calibration standard.

Number	Area (mAU)
3.1	216,121
3.2	215,065
3.3	214,128
3.4	217,360
3.5	216,388
3.6	215,81
Average	215,810
STD	1,12
RSD	0,52 %

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 7: Limit of detection for gallic acid.

	MWD detector
Height noise (H1)	0,037 mAU
Peak height lowest calibration standard (H2)	2,4 mAU
Concentration lowest calibration standard (C)	0,36 ppm
LoD ((3*H1)/H2*C)	0,0167 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 8) show a carryover effect of less than 2% and is therefore acceptable.

Table 8: Carryover effect of the method.

	MWD detector
Peak height blank (H3)	0,251 mAU
Peak height highest calibration standard (H4)	228,0 mAU
Carryover effect (H3/H4*100%)	0,110%

INJECTION VOLUME

The injection volume accuracy of the autosampler was established based on two injections. The peak areas of two samples, with respectively concentrations of 1,5 and 6 ppm and injection volumes of 20 µl and 5 µl, were compared. The relative standard deviation of the injection volume is 1,9% and is therefore acceptable. The results are shown in Table 9.

Table 9: Accuracy of the injection volume.

	MWD detector
Peak area 20 µl, 1,5 ppm solution	109,607 mV
Peak area 5 µl, 6 ppm solution	111,68 mV
Standard deviation (STD)	2,073 mV
Relative standard deviation (RSD)	1,9%

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 4 and Table 10, the pressure ripple for this method is 0,50%.

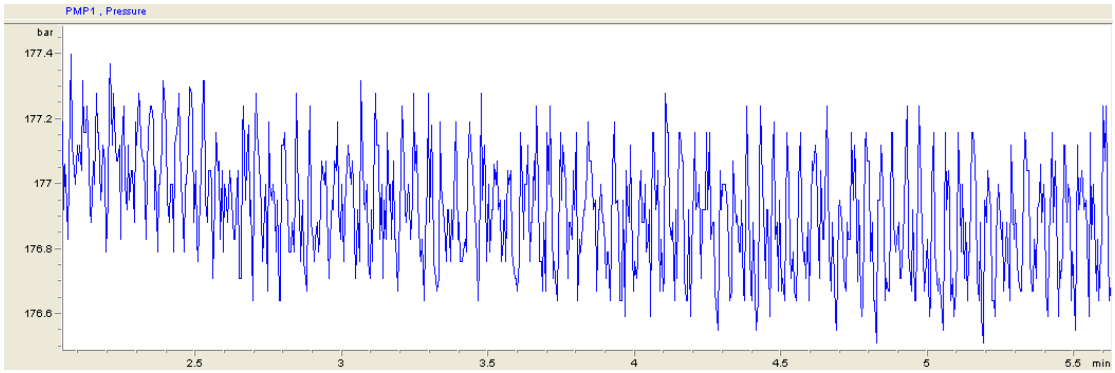

Figure 4: Display of pressure ripple during a run.

Table 10: Pressure ripple.

	Pressure
Highest pressure	177,40 bar
Lowest pressure	176,52 bar
Standard deviation (STD)	0,88 bar
Relative standard deviation (RSD)	0,50%

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.

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