

# TEST CERTIFICATE

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S-A-LE 326



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INSTRUMENT: HPLC - DAD  
ID NR.: 326  
MODEL: AGILENT SERIES 1100  
CAL. DATE: 5 SEPTEMBER 2017

# HPLC - DAD

NR. 326: AGILENT SERIES 1100

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## SYSTEM TESTS

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In this report the performance of the Agilent Series 1100 HPLC system was investigated. The system consisted of a degasser, binary pump, autosampler, column oven compartment and a DAD detector. 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	:	DAD, 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.

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**SYSTEM PERFORMANCE**

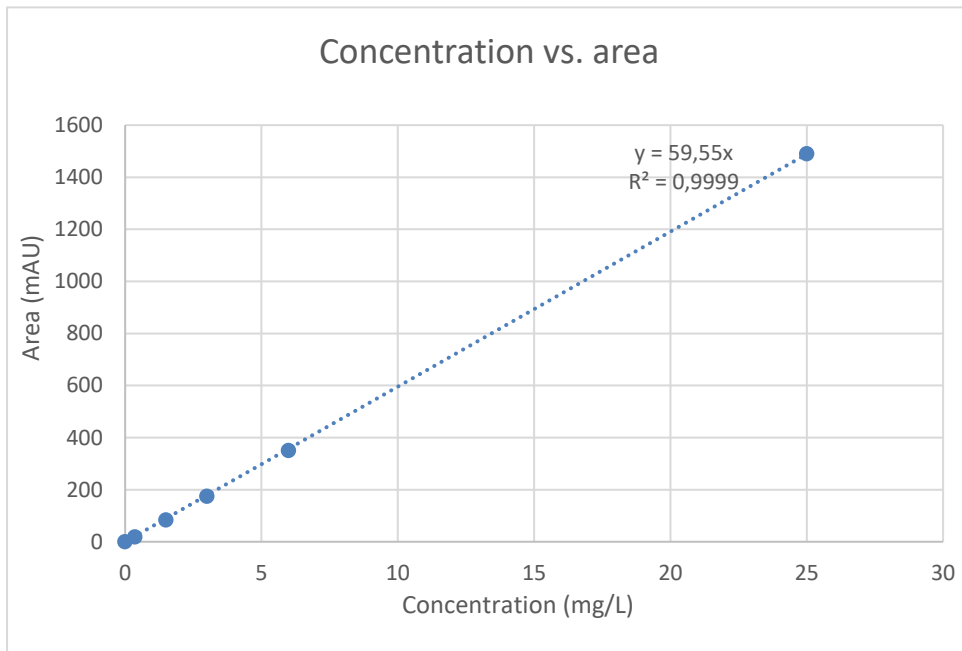
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**LINEARITY**

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 1 below. The correlation coefficient of 0,9999 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	19,2
1,5	84,5
3,0	175,7
6,0	351
25,0	1491



*Figure 1: 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 (2,30%) is within the acceptance criterion of 5,0% and therefore acceptable.

*Table 2: Repeatability of the mid cal. standard*

Number	Area (mAU)
3.1	173,9
3.2	175,2
3.3	175,7
3.4	173
3.5	175,8
3.6	175,6
3.7	176,1
3.8	175,5
Average	175,2714286
RSD (%)	0,61

### DETECTION LIMIT

An example of the obtained chromatogram at the lowest calibration standard of 0,36 mg/l is presented below in Figure 2. From this chromatogram the detection limit (3x noise) was determined. The obtained detection limit was 0,036 mg/l for this substance.

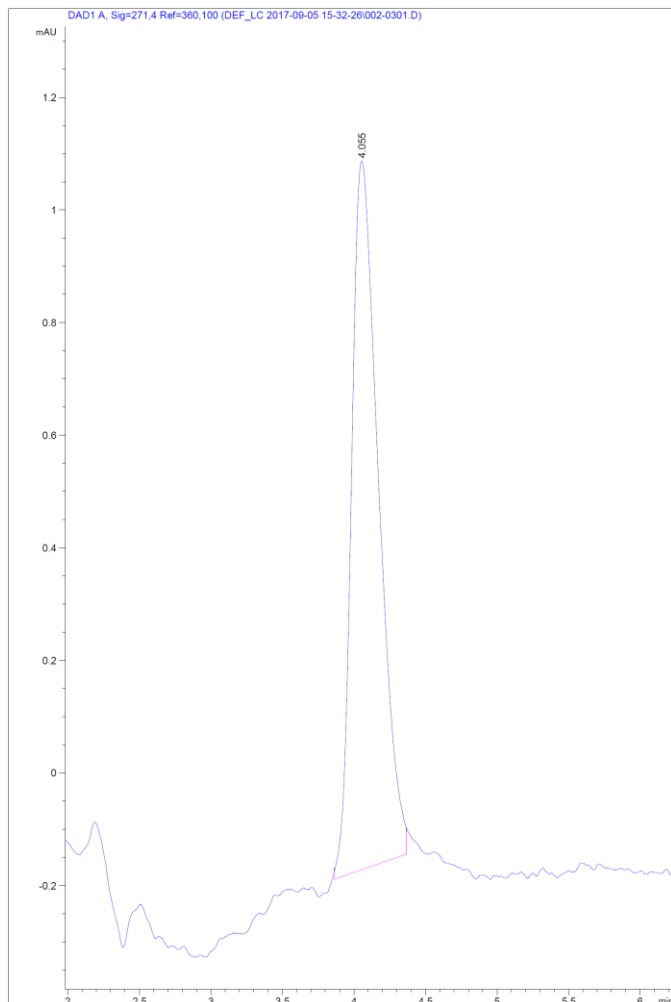


Figure 2: Obtained chromatogram lowest standard

### LEAK TEST

In order to assure the performance of the binary pump as final check-up an internal leak test was conducted. The pressure in the pump showed a good linearity and the leak test passed the internal criteria. The result of the executed leak test are presented below in figure 3.

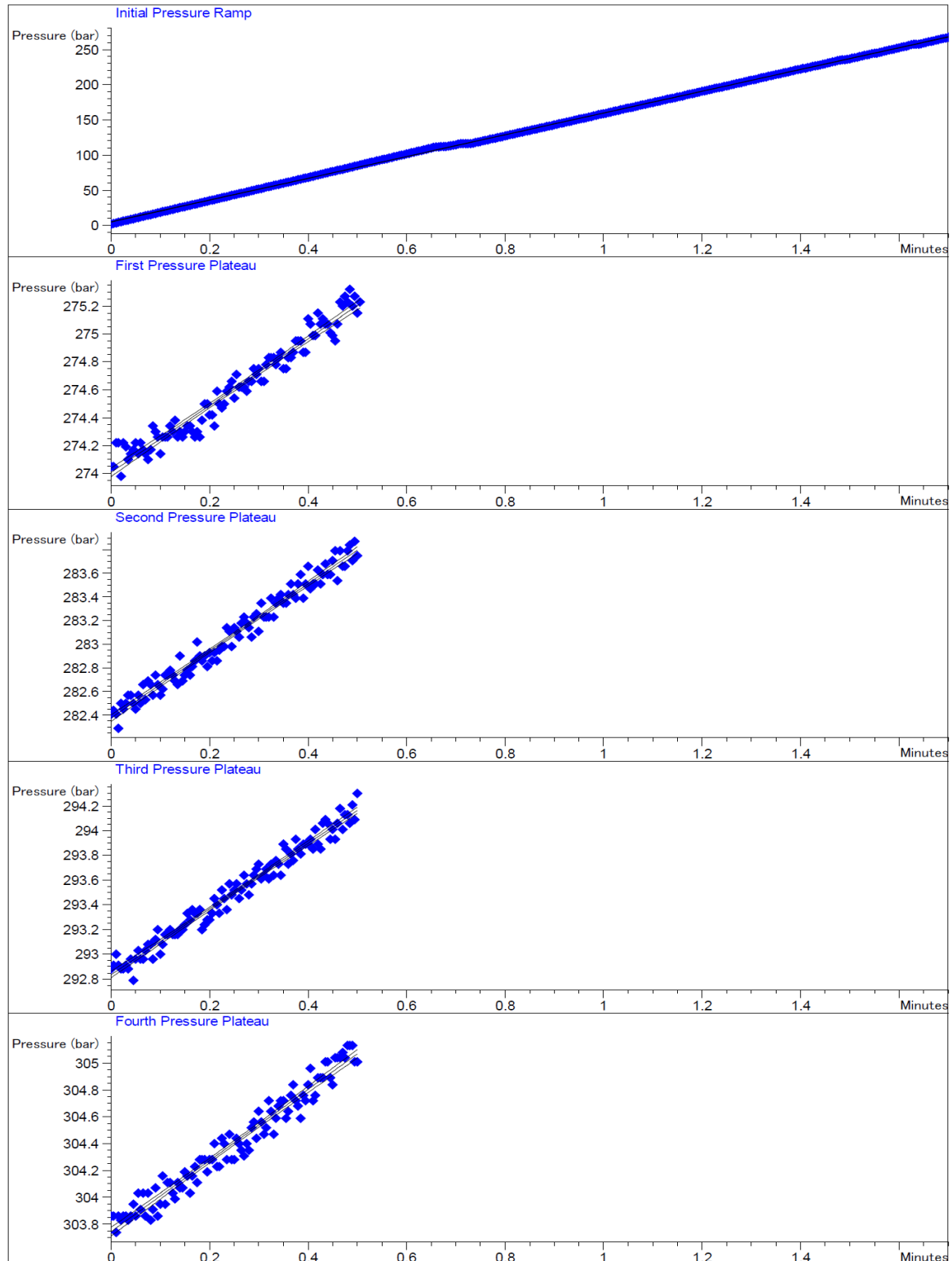


Figure 3: Result of the executed leak test

Calibration certificate:  
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**CONCLUSION**

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

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**AUTHORISATION: 5-9-2017**

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Date: 5-9-2017