

TEST CERTIFICATE

S-A-LE 1389



DORSHORST 2
7217PH HARFSEN
NETHERLANDS

INSTRUMENT: HPLC – UV/VIS - FLD
ID NR.: 1389
MODEL: SHIMADZU PROMINENCE LC-20
CAL. DATE: JANUARY 13, 2021

HPLC - UV/VIS - FLD

NR. 1389: SHIMADZU PROMINENCE LC-20

GENERAL

In this report the performance of the HPLC system, of combined Shimadzu prominence LC-20 modules, was investigated. The system consisted of two reservoir trays, degassing unit, solvent delivery unit with gradient valve, autosampler, column oven, UV/Vis detector, fluorescence detector, communications bus module and PC with LabSolutions software version 5.54.

Table 1: System specifications

Module	Model	Serial
Degassing unit	DGU-20A 5R	L20705061479 IX
Prominence liquid chromatograph pump	LC-20AT	L20115174195 US
with gradient valve kit	LC-20AD/T LPGE KIT	L20265161662 US
Prominence autosampler	SIL-20A HT	L20345170467 US
Prominence column oven	CTO-20AC	L20215175407 US
with integrated column switching valve	FCV-14AH	C20455002506 CD
Prominence UV/Vis detector	SPD-20A	L20135174618 US
Prominence fluorescence detector	RF-20A XS	L20505173347 US
Communications bus module	CBM-20A	L20235176231 US

The system was tested in practice to check the operational performance of the instrument modules and software. For this, a test application to Gallic acid by HPLC-UV/VIS-FLD has been developed and used.

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 used:

- HPLC column : Atlantis dC18, 3 μ m 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 μ l
- UV/VIS detector : Wavelength 271 nm
- Fluorescence detector : Excitation wavelength: 278 nm, emission wavelength: 360 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 with the UV/VIS detector and fluorescence detector. The five-point calibration curve of Gallic acid standard solutions was recorded with the UV/VIS detector and fluorescence detector.

UV/VIS detector

The results of the analysis are shown in Table 1 and Figure 1 below. The correlation coefficient of 1 comply with the specified acceptance criterion of $r > 0,999$.

Table 1: Results of the five-point calibration curve

Concentration (ppm)	Area (mV)
0,36	27472
1,5	117645
3	239295
6	483768
25	2010464

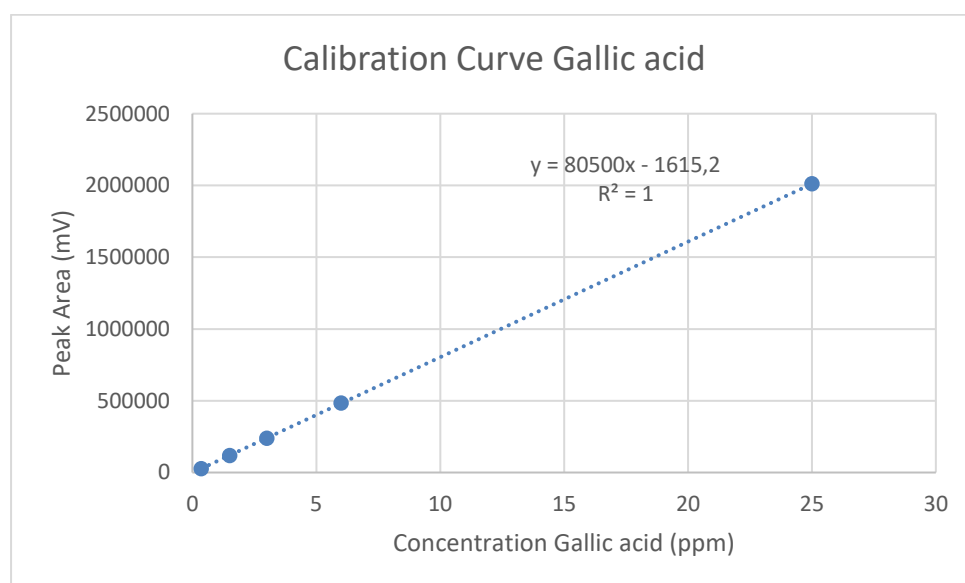


Figure 1: Graph of the five-point calibration curve with the UV-VIS detector.

Calibration certificate:
S-A-LE 1389

Fluorescence detector

The measured values of the curve are shown in Table 2 and Figure 2 below. The correlation coefficient of 0,9991 comply with the specified acceptance criterion of $r > 0,999$.

Table 2: Results of the five-point calibration curve

Concentration (ppm)	Area (mV)
0,36	18137
1,5	70238
3	128047
6	224846
25	828972

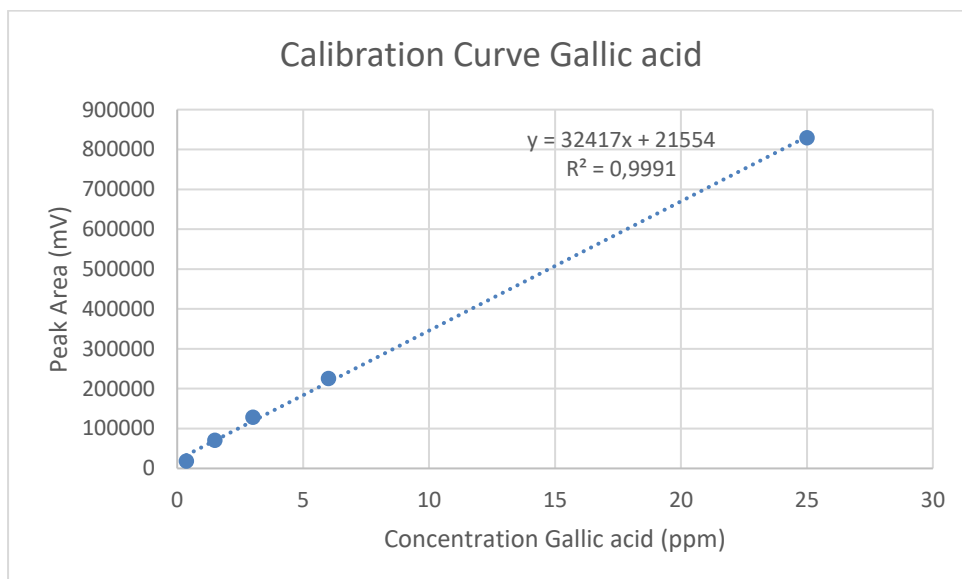


Figure 2: Graph of the five-point calibration curve with the fluorescence detector

REPEATABILITY

The repeatability was determined for the mid calibration standard with a concentration of 3 ppm gallic acid. The results are presented in Table 3 for the UV/VIS detector and in Table 4 for the fluorescence detector. The relative standard deviation of both is within the acceptance criterion of 5,0% and therefore acceptable.

Table 3: Repeatability of the mid calibration standard (UV/VIS detector)

Number	Area (mV)
3.1	239824
3.2	239509
3.3	239196
3.4	238787
3.5	239034
3.6	238872
Average	239203,67
STD	397,62
RSD	0,17 %

Table 4: Repeatability of the mid calibration standard (fluorescence detector)

Number	Area (mV)
3.1	119319
3.2	123512
3.3	125352
3.4	123014
3.5	121547
3.6	126486
Average	123205
STD	2582,84
RSD	2,1%

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 5: Limit of detection for gallic acid.

	UV/VIS detector	Fluorescence detector
Height noise (H1)	0,323 mV	0,0658 mV
Peak height lowest calibration standard (H2)	2409 mV	1231 mV
Concentration lowest calibration standard (C)	0,36 ppm	0,36 ppm
LoD $((3*H1)/H2*C)$	0,00014 ppm	0,000058 ppm

CARRYOVER EFFECT

The carryover effect of the method was determined based on the peak height of samples when a blank solvent was injected directly after the highest calibration standard. The results show a carryover effect of less than 2% and are therefore acceptable.

Table 6: Carryover effect of the method.

	UV/VIS detector	Fluorescence detector
Peak Height Blank (H3)	27601 mV	63348 mV
Peak height highest calibration standard (H4)	0,139 mV	132 mV
Carryover effect (H3/H4*100%)	0,001%	0,21%

INJECTION VOLUME

The injection volume accuracy of the autosampler was established with two injections using the UV/VIS detector. The peak areas of two samples with respectively concentrations of 1,5 and 6 ppm and injection volumes of 20 µl and 5 µl. The relative standard deviation of the injection volume is 1,8% and is therefore acceptable. The results are shown in Table 7.

Table 7: Accuracy of the injection volume.

	UV/VIS detector
Peak area 20 µl, 1,5 ppm solution	114664 mV
Peak area 5 µl, 6 ppm solution	116753 mV
Standard deviation (STD)	2089 mV
Relative standard deviation (RSD)	1,8%

PRESSURE RIPPLE

The performance of the pump is assessed by determining the stability of the pressure in operation. The pressure ripple may have a maximum deviation of 1%. As shown in Figure 3 and Table 8, the pressure ripple for this method is 0.69%.

Calibration certificate:
S-A-LE 1389

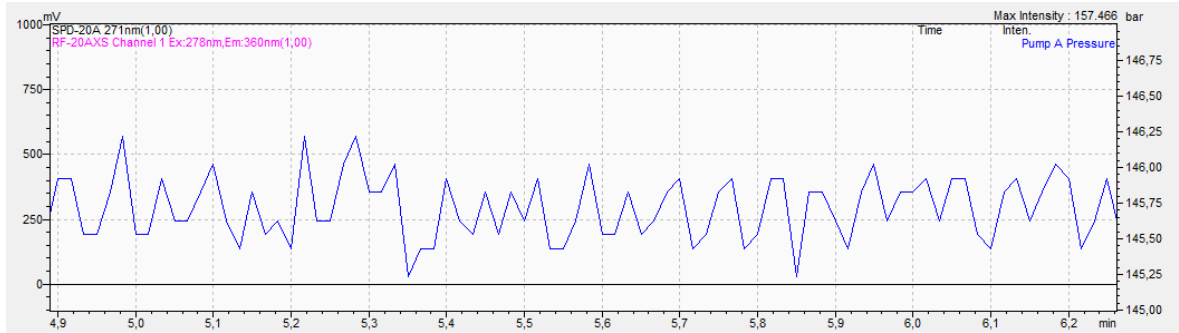


Figure 3: Display of pressure ripple during a run.

Table 7: Pressure ripple.

	Pressure
Highest pressure	146,25 bar
Lowest pressure	145,25 bar
Standard deviation (STD)	1,00 bar
Relative standard deviation (RSD)	0,69%

Calibration certificate:
S-A-LE 1389

CONCLUSION

The system meets all the stated criteria and is therefore in a good working state.

AUTHORISATION: 13-01-2021



H. Kerkdijk
Director S-A-LE

Date: 13-01-2021