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Pump Linearity and Dwell Volume Measurements

Protocols for LC Instruments

2020

How is the Linearity of the Pump Measured?

The linearity of the pump should be assessed between 0-100% using the conditions described in *Figure 1*. This is in order to ascertain the ability of the pump to accurately proportionate the mobile phase throughout the gradient, but in particular, at low or high percentages. Proportionating at low or high concentrations puts the most strain on the pumping mechanism.

The method uses a simple mobile phase of water in Line A and uracil dissolved in water as the B mobile phase as the compound has a UV chromophore which can provide a linear response with increasing concentration. The chromatogram produced is a linear response which corresponds to the changing gradient and can highlight any deviation in the pumping mechanism. This can be easily emphasised by adding a line between the top and bottom of the gradient.

Method Conditions

Sample:	Water	
Mobile Phase A:	Water	
Mobile Phase B:	5 mg/L Uracil in water	
Gradient:	Time	%B
	0.0	0
	10.0	100
	12.0	100
	12.5	0
	15.5	0
Flow Rate:	2 mL/min	
Column:	Zero dwell volume (ZDV) union	
Injection Vol.:	0.5 µL	
Oven Temp.:	40 °C	
Run Time:	15.5 mins	
Number Inj.:	3	
Sampling Frequency:	12.5 Hz	

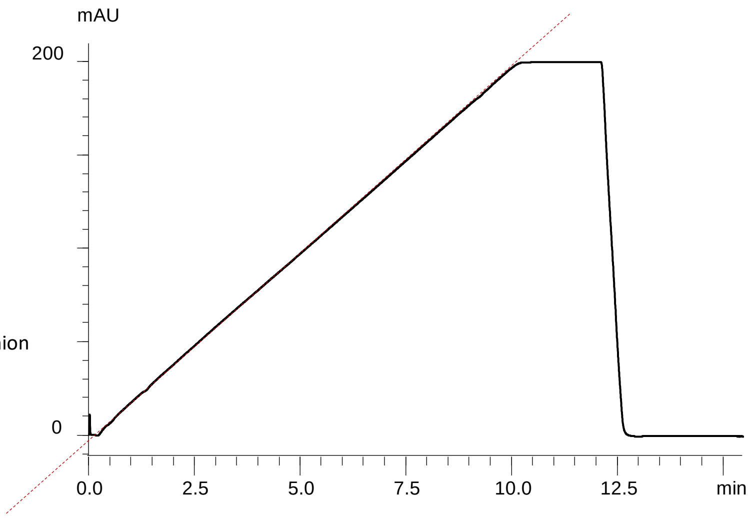


Figure 1 Operating conditions for the pump linearity test and a corresponding chromatogram with a line to illustrate linearity throughout the entire gradient.

Ideally, the response should be linear throughout the gradient, however, it has been observed that some pumps deviate at the upper and lower limits, as illustrated in *Figure 2*. This could potential cause issues when methods are transferred between instruments with different pump designs.

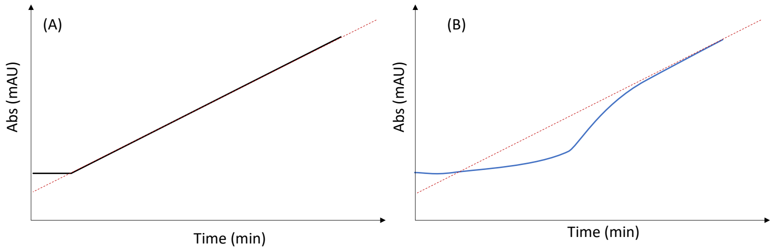


Figure 2 Schematic of (A) the linear response at the lower percentage composition and (B) the deviation of the pump linearity at the lower percentage composition.

How is Dwell Volume Measured?

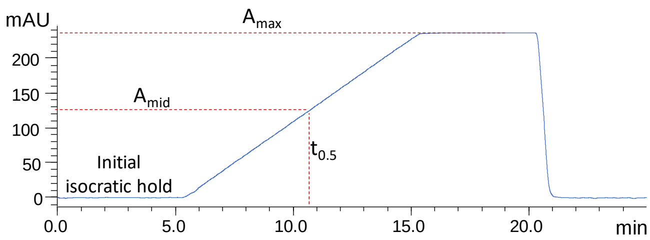
Dwell volume (sometimes referred to as delay volume) is calculated from the point where two solvents first mix to the head of the column. This encompasses the tubing, mixer, autosampler and column switch, if installed. Determining the dwell volume is one of the most essential tests which should be performed to understand an LC system as it differs between each system and plays an important role in gradient chromatography.

A gradient is a continuous change in mobile phase. However, the change is not instantly observed by the column due to the delay from where the mobile phase first meet to the column head. It can be measured using the conditions described in *Figure 3*, and hence calculated using the defined equations.

The %B range and flow rate should be representative of typical conditions used on the LC system. For example, a HPLC system typically uses 1 mL/min with 10-90%B over 15 minutes. The %B/min change is 80%B/15 mins which equates to 5.3%B/min. Thus, in the dwell volume test with a t_G of 10 minutes, the %B should cover the range of 53% i.e. 10-63%B over 10 minutes.

Method Conditions

Sample:	Water
Mobile Phase A:	Water
Mobile Phase B:	5 mg/L Uracil in water
Gradient:	Time / %B
	0.0 / 10
	5.0 / 10
	15.0 / 63
	20.0 / 63
	20.5 / 10
	25.5 / 10
Flow Rate:	1 mL/min
Column:	Zero dwell volume (ZDV) union
Injection Vol.:	0.5 µL
Oven Temp.:	40 °C
Run Time:	15.5 mins
Number Inj.:	3
Sampling Frequency:	12.5 Hz



Description	Measurement
Endpoint of the gradient (A_{max}):	Measured in processing software
Midpoint of the gradient (A_{mid}):	$A_{max}/2$
Midpoint of the gradient ($t_{0.5}$):	Use A_{mid} value to determine $t_{0.5}$ in processing software
Dwell time (t_D):	$t_{0.5} - t_G/2$
Dwell time corrected ($t_{D(corr)}$):	$t_{D(corr)}$ – *initial isocratic hold*
Dwell Volume (V_D):	$t_{D(corr)}$ X F

Figure 3 Experimental conditions and calculations to measure dwell volume.

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The two main benefits for determining dwell volume are:

- Selecting appropriate column formats and gradient conditions for the LC system.
    - It would be impractical to use small length, narrow bore columns or ballistic gradients on an LC system with significant dwell volume. This is due to the effective isocratic delay caused by the dwell volume which can be detrimental for a separation.
- Translating gradient methods between different LC instruments.
    - The changing gradient composition will reach the column at different times on two LC systems with varied dwell volumes if directly compared. This can have a fundamental influence on selectivity with differences in the chromatographic elution profile, and the potential to change resolution between critical pairs.
      However, if the dwell volume between two instruments are known, the methodology can be translated in order to maintain selectivity. This is done by either introducing an isocratic hold or the injection is delayed to simulate the dwell volume of the original system. This is covered in greater detail in “A Quick Guide to Translate Gradient Methods”.

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