How to Determine System Dwell Volume Theory and Practice

AKN0001

ACE Knowledge Note #0001

Introduction

Determining the dwell volume of an LC system is a useful activity and important for gradient method transfers between different instruments; to maintain peak retention, selectivity and resolution. This technical note describes the theory behind dwell volume and how to measure it accurately.

Dwell Volume

The dwell volume, or gradient delay volume, is described as the system volume between the point at which eluents are combined (in the pump mixer) and the head of the column.

Dwell volumes differ considerably depending on the configuration of the instrument, the vendor, the volume of tubing, binary or quaternary pumps and if any mixers are installed. Typical instrument dwell values can be seen in Table 1.

Dwell volume considerations are less important for isocratic analyses as the mobile phase composition remains constant.

For gradient chromatography it is different. The volume between initial eluent mixing and the point at which the mixed eluent reaches the column can have significant impact on selectivity and retention. Moving gradient methods between instruments can easily result in different chromatography (retention times, peak elution order) as a result of dwell volume differences.

Understanding the dwell volumes for your instruments is therefore good practice to ensure seemless gradient method transfers between instruments.

Instrument	Typical Dwell Volume (µL)
Agilent HP1100 Binary	180-900
Agilent HP1100 Quaternary	800-1100
Agilent 1200 RRLC	~300
Dionex P680A Quaternary	<400
Thermoquest P4000 Quat	<600
Waters Alliance 2695 Quat	600
Waters Varian 9012 Ternary	1000
Waters Acquity UPLC	~100

Table 1 Typical dwell volume values for different instrument manufacturers

Methodology

The dwell volume may be determined as follows:

1. Replace the column with a Zero Dead Volume (ZDV) connector.
2. Place water on Solvent Line A and water containing 0.1% v/v acetone on Solvent Line B.
3. Monitor the run using UV detection at 265 nm.
4. Run the gradient program in Table 2 at 2 mL/min.

Time	%B
0	0
10	100
12	100
12.5	0
Post: 3 mins	

Table 2 Dwell volume gradient

The resulting chromatogram is seen in Figure 1. To identify the midpoint of the gradient ($t_{0.5}$), subtract the UV absorbance at the end of the gradient (A_{max}) from the UV absorbance at the beginning of the gradient (A_{min}). This is divided by two and the time of $A_{0.5}$ is determined. The dwell time is calculated as:

$$t_D = t_{0.5} - \left(\frac{t_G}{2}\right)$$

where t_D is dwell time (min) and t_G is gradient time (min). Dwell volume is then calculated as:

$$V_D = t_D \times F$$

where F is the flow rate (mL/min)

System Dwell Volume	
Gradient endpoint (A_{max})	123.138 mAU
Gradient midpoint ($A_{0.5}$)	61.569 mAU
Gradient midpoint ($t_{0.5}$)	5.566 min
Dwell time $t_D = t_{0.5} - \left(\frac{t_G}{2}\right)$	0.566 min
Dwell volume $V_D = t_D \times F$	1.13 mL

Table 3 Worked example to calculate dwell volume

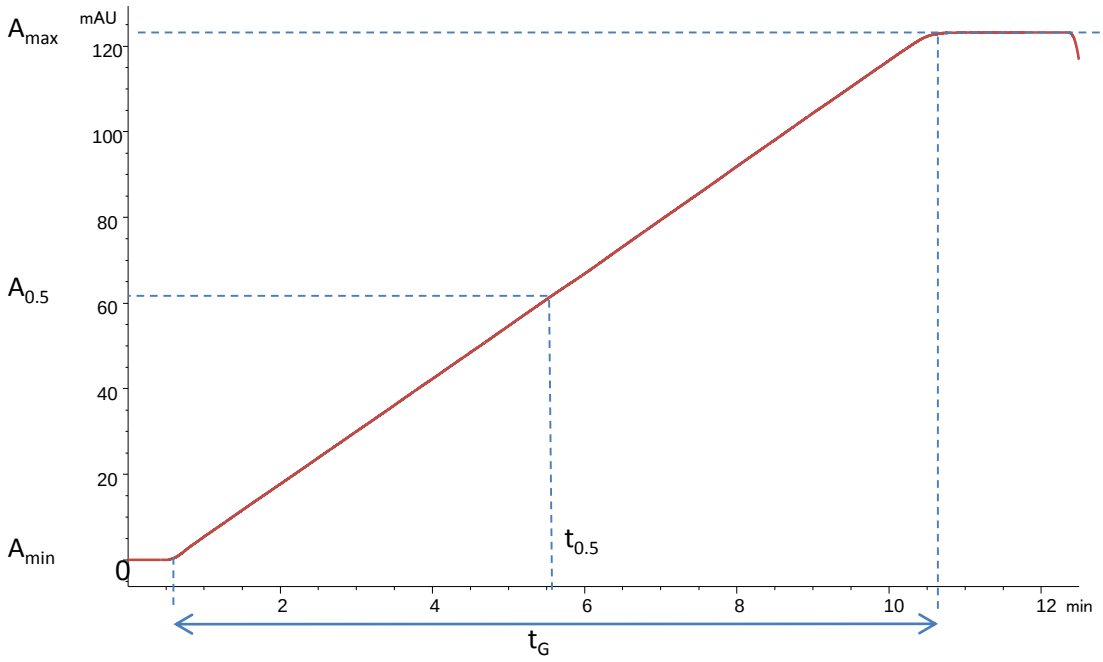

Figure 1 Example of chromatogram produced to calculate dwell volume

Conclusion

A simple, generally applicable method for determining dwell volume in gradient chromatography has been described.