Chromatography Solutions

Knowledge note #0001

How to Determine System Dwell Volume: Theory and Practice

INTRODUCTION

Determining the dwell volume of an LC system is a useful activity and important for gradient method transfers between different instruments; to maintain analyte retention, selectivity and resolution. This technical note defines the system dwell volume and demonstrates how to accurately quantify it.

DWELL VOLUME

The dwell volume (V_D), or gradient delay volume, is defined as the volume of the fluidic path between the point at which eluents are combined (in the pump mixer) and the head of the column. This includes volumes attributable to LC pump components such as mixers, valves and system tubing, along with autosampler components.

Dwell volumes differ considerably between LC systems and depend on the instrument configuration, the volume of tubing, whether a binary or quaternary pump is installed and if any mixers are present. Examples of typical instrument dwell values are presented in Table 1.

Table 1: Typical dwell volume values for a range of LC 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

Dwell volume considerations are relatively unimportant for isocratic analyses, as the mobile phase composition remains constant throughout the analysis.

For gradient chromatography however, the dwell volume is important. 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. Transferring gradient methods between instruments, or translating methods to different column formats, can often result in different chromatography (retention times, peak elution order) as a result of dwell time differences.

Understanding and determining the dwell volumes of your instruments is therefore good practice, to ensure accurate gradient method transfers between instruments.

DWELL VOLUME DETERMINATION

The dwell volume may be measured 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 following gradient program with a flow rate of 2 mL/min

Time	%B
0	0
10	100
12	100
12.5	0

Post time: 3 mins

The resulting chromatogram is shown in Figure 1. To identify the midpoint of the gradient ($t_{0.5}$), subtract the UV absorbance at the beginning of the gradient (A_{min}) from the UV absorbance at the end of the gradient (A_{max}) to determine the absorbance midpoint ($A_{0.5}$).

The corresponding time value ($t_{0.5}$) can then be used to determine the dwell time as follows:

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

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)

WORKED EXAMPLE

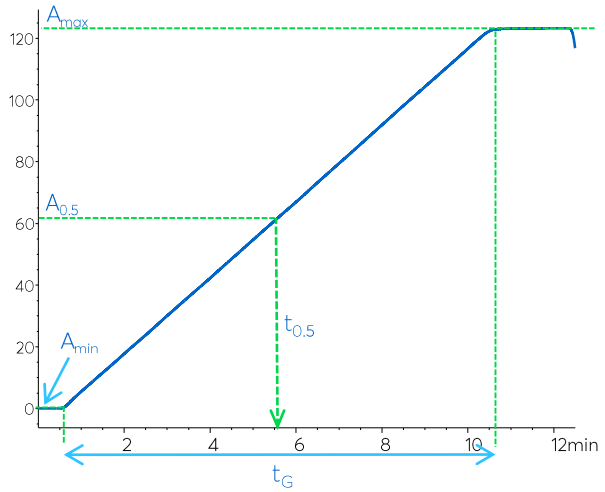

Figure 1: Example chromatogram for dwell volume determination

A_{max} - A_{min}	123.138 mAU
Absorbance midpoint ($A_{0.5}$)	61.569 mAU
Gradient midpoint ($t_{0.5}$)	5.566 min
Dwell time, t_D	0.566 min
Dwell volume, V_D	**1.13 mL**