Waters
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Large Volume Injections
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LARGE VOLUME INJECTIONS FOR IMPROVED LC SENSITIVITY

It is important to realize that often a large sample injection (e.g. 200 µl) will experience only a little additional band broadening over that which would occur for a small injection (e.g. 10 µl). Thus, the large injection can result in a higher concentration for detection with no loss in resolution.* Also, improved chromatographic precision can result if there is smaller uncertainty of measurement with the larger volume injection.

A rule of thumb is that the injection volume can be as high as 30% of the volume of a peak which elutes from the column when using a small injection (e.g. 10 µl) and there should be no significant broadening of the peak with this larger injection. This rule of thumb is understood by considering the contributions to band broadening.

At injection, the sample volume will be diluted to a volume which is mainly dependent upon the high performance column. The final peak volume width which is observed in the detector is a result of the volume of the sample injected and of the spreading from the column, the detector, and the extra column effects.

$$(1)\quad W^2_{peak} = W^2_{Inj} + W^2_{Col} + W^2_{Det} + W^2_{Ex}$$

Dilution in the chromatographic system is defined simply by the efficiency of the column and can be calculated from the plate equation:

$$(2)\quad N = 16(V_e/W_{peak})^2$$ where V_e is elution volume, N is the plate number, and W_{peak} is the base width determined by the "tangent" meth

Rearranging gives

$$(3)\quad W^2_{peak} = 16\ V_e^2/N$$

Using equations (1), (2) and (3) we can calculate the effect of large volume injections.

To use an example, let's assume we have two columns, one with a plate count of 25,000, a 10 ml retention volume,and the spreading is W=0.8 ml and another one with an 8000 plate column, a 10 ml retention volume, and the spreading is W=0.45 ml For these two situations the effect of injection volume size can be shown in Table

* This assumes there is no mass overload which is often a valid assumption if there is insufficient sensitivity. However, experimentally this hypothesis should be checked for each situation.

See reverse side for Table 1.

TABLE 1

Influence of Injection Volume on Peak Volume

2500 Plate Column			8000 Plate Column		
W_{inj}	W_{col}	W_{peak}	W_{inj}	W_{col}	W_{peak}
0.01 ml	0.80 ml	0.80 ml	0.01 ml	0.45 ml	0.45 ml
0.1 ml	0.80 ml	0.81 ml	0.1 ml	0.45 ml	0.46 ml
0.2 ml	0.80 ml	0.82 ml	0.2 ml	0.45 ml	0.49 ml

When the plate count of the column was 2500 an injection volume size of 200 µl is easily tolerated and the larger injection volume increased the peak height by 20 times over the 10 µl injection and increased the peak width only 2.5%. Thus, the increased sensitivity is a "free" benefit. For the higher plate column, a 200 µl injection may still be satisfactory since it only broadens the peak by an additional 9%.

Most people dismiss large volume injections. However, experienced chromatographers realize when to use larger volume injections to help enhance sensitivity and improve precision.

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