How to Determine Extra Column Dispersion and Extra Column Volume

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The width of a chromatographic peak is dependent on various dispersion processes operating both inside and outside the column. Extra column volume (ECV) is an important source of extra column dispersion (ECD) and can have significant detrimental effects, resulting in losses in column efficiency and therefore peak resolution. This Knowledgebase article outlines how ECV and ECD can be easily measured.

Introduction

When an analyte is injected onto an LC instrument, it migrates through the system and column in a discrete band. The measured width of the band is affected by dispersive processes occurring within the column packed bed (intra-column) and within the LC system components (extra-column). The total observed peak dispersion (σ^2_{tot}), defined as the variance of a Gaussian shaped peak, can be expressed as follows:

$$\sigma^2_{tot} = \sigma^2_{col} + \sigma^2_{ext}$$

Where σ^2_{col} and σ^2_{ext} are the contributions from intra-column dispersion and extra-column dispersion (ECD) respectively.

Whilst intra-column dispersion is linked to the column, ECD has two contributors that may be optimised by the chromatographer. Extra column volume (ECV) is comprised of the internal volumes of various system components including tubing, fittings, autosampler and detector flow cell and is a major contributor to ECD. If ECV is large, then significant band broadening may occur, resulting in wider peaks and a loss in efficiency and resolution. Time- related factors such as detector sampling rate also affect the observed peak width. It is important to ensure detector settings are set to acquire enough data points to accurately describe the peak. This Knowledgebase article explains how ECV and ECD can be easily determined for any LC system.

The influence of ECV is generally insignificant for large volume columns (e.g. 150 x 4.6 mm). However, for smaller volume columns (e.g. 50 x 2.1 mm), the effects of ECV are more pronounced. Applications utilising small columns packed with small particles therefore require LC systems with highly optimised ECV, i.e. UHPLC systems; although it is possible to substantially reduce the ECV in many standard LC systems. Figure 1 shows the same isocratic separation run on a 75 x 3.0 mm column on systems with low and high ECV. Even for this moderate dimension column, the impact of ECD on resolution and efficiency is clearly evident and would be more pronounced on a smaller i.d. column.

If excessive ECD is suspected as the cause of poor method performance, ECV should be reduced where possible or the method transferred to a more optimised system. Alternatively, the method can be translated to a column with a larger i.d. to minimise the impact of ECV. As mentioned, data capture rate should also be optimised, particularly for very fast separations, or those using small volume columns. An insufficient data capture rate can result in poor description

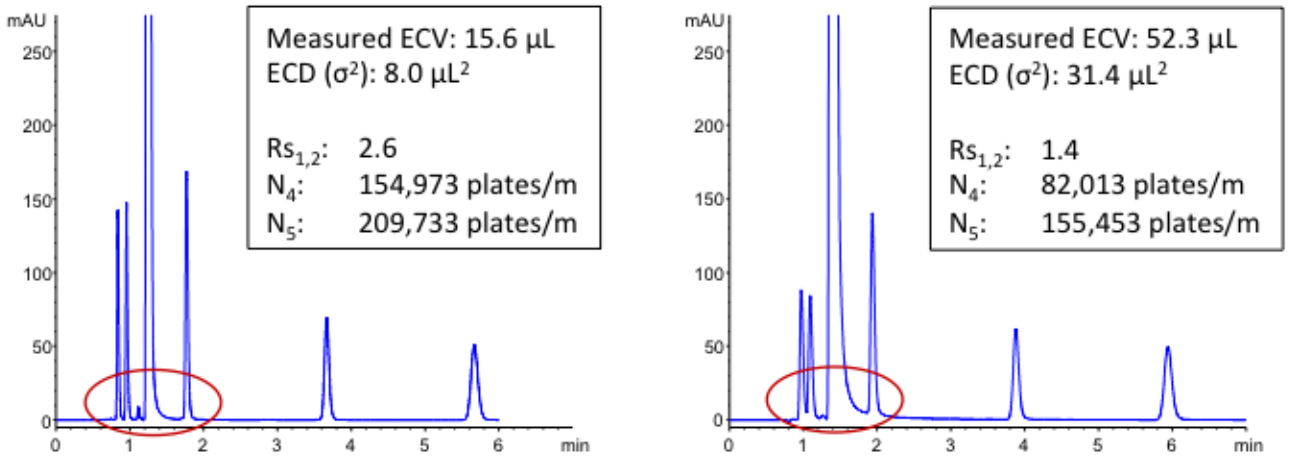

Figure 1. Comparison of the separation of aspirin and related substances on a 2.5 µm core shell C18 75 x 3.0 mm column using LC systems with low and high ECV and ECD.

of chromatographic peaks, resulting in artificially broad peaks and loss in efficiency.

It is useful to experimentally determine both ECV and ECD for LC systems in order to ensure that the appropriate column format is utilised. The ECV and ECD can easily be determined using the following procedure.

ECD can have a large negative impact on column performance, particularly when performing separations on smaller volume columns. ECV is a major contributor to ECD. Experimental determination of the ECV and ECD of any LC system allows the chromatographer to select a suitable column format for their application, in order to maximise efficiency and achieve optimum chromatography.

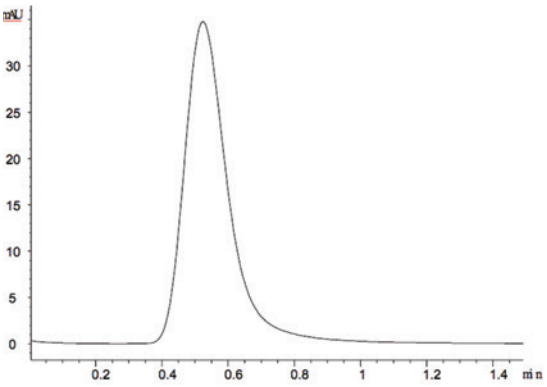

Figure 2. Example of chromatogram produced from experimental method.

Conclusion

Experimental

Mobile phase: methanol/water (49:51 v/v).

Sample: 1% acetone in methanol/water (49:51 v/v).

1. Replace the column with a Zero Dead Volume (ZDV) connector. Set the column oven temperature to 40 °C.
2. Set the flow rate to 0.1 mL/min.
3. Set the UV detector to 254 nm and set the data capture rate to maximum.
4. Once equilibrated, perform at least six replicate 0.5 µL injections. Figure 2 shows a typical chromatogram.
5. Record the retention time and the efficiency at half height, $N_{0.5}$.
6. The extra column volume is estimated by using the following equation:

$$ECV = t_R \times F$$

Where t_R is retention time in minutes and F is the flow in µL/min.

7. ECD is determined as follows:

$$ECD = \sigma^2$$

$$\text{where } \sigma = \frac{(t_R \times F)}{\sqrt{N_{0.5}}}$$

8. Calculate the average of six injections and then determine %RSD. The %RSD should be less than 2%.