Translations Between Differing Formats of Liquid Chromatography: Advantages, Principles, and Possible Pitfalls

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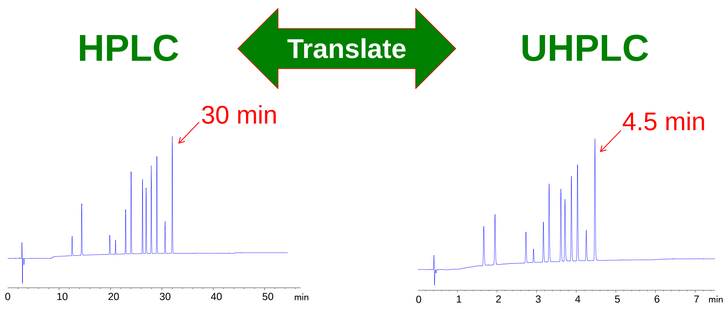
Translating LC Methods



Interest in chromatographic method translation has been increasing since the introduction of commercially available UHPLC instrumentation [1,2] and new column formats. [3-5] This has resulted in increased resolution through the reduction of stationary phase particle size; introduced faster analyses with shorter run times; and significantly reduced solvent consumption. With the use of standard HPLC systems expected to decline [6], there is a real need to be able to perform reliable and accurate translations between differing LC formats.

Other drivers for method translations include differing access to equipment, lack of experience in different organizations with new techniques, and equipment updates.

A variety of free and commerical translation tools exist to assist chromatographers in the translation of methods. While, in principle, translations obey well defined chromatographic theories that are well documented [refs. available in 7], there are a number of potential pitfalls that may result in poor translations and, consequently, selectivity differences and failure to meet resolution system suitability criteria.

This poster discusses factors that impact the accuracy of LC method translation and describes a new tool that addresses these issues and results in translations with increased accuracy.

Potential Pitfalls

Dwell Volume Differences

Dwell volume (V_D)— the volume from the point where two solvents (A and B) first meet, to the inlet of the column.

Accurate translation of gradient methods requires the ratio between dwell volume and dead volume to be kept constant. Significant differences impact column selectivity and relative retention time shifts which may lead to co-elution of peaks and in some cases peak reversal.

A series of non-steroidal anti-inflammatory drugs (NSAIDs) were chromatographed on the same column using a binary high pressure mixing system with low dwell volume (202 µL)—System A; and a quaternary low pressure mixing system with a higher dwell volume (990 µL)—System B. In this case a translation from V_D/V_M = 1.8 (Fig. 1A) to a system with V_D/V_M = 9 (Fig. 1B) resulted in reversal of elution order (peaks 3 and 4) and co-elution (peaks 7 and 8).

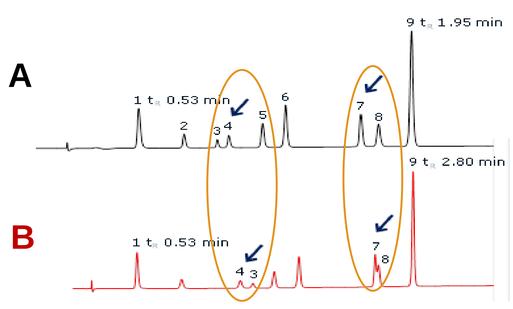


***Figure 1**: A change in dwell volume: dead volume ratio from 1.8 in system A (at high pressure), to 9 in system B (at low pressure) for a mixture of NSAIDs.*

In practice, differences in dwell volume can be compensated for. In this case an injection delay was implemented in the low pressure system (C) to compensate for the V_D difference. This led to both the original selectivity and relative retentions being maintained (Fig. 2).

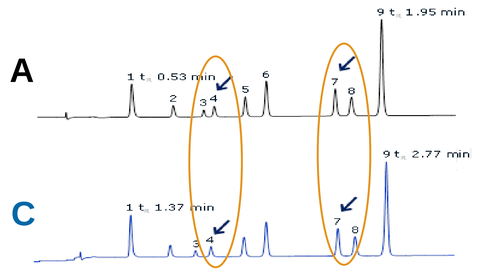


***Figure 2**: Injection delay in system C (low pressure) maintains relative retention and selectivity of a chromatographed NSAID mixture.*

Due to the importance of dwell volume in method translations, all gradient methods should state the dwell volume of the instrument used to develop the methodology.

Incorrect Dead Volume Estimation

Dead Volume (V_M)—the volume of mobile phase required to elute an un-retained compound from the column.

Column dead volume is a fundamental parameter that has a big impact on translations and their accuracy. Currently available translators, however, erroneously assume equal porosity for stationary phases.

Standard equations can be used to estimate V_M based on superficially porous particles using reported particle and core diameters. Due to the variety of methods used to determine and report particle size, however, the error associated with these estimates can be substantial. [7] In addition parameters like particle pore size and pressure differences when packing columns affect porosity. Difference between theoretical and measured dead volumes, therefore, can be substantial (up to approx. 30% [7]).

The chromatograms in figure 4 were generated using the same column, solvents, and sample. The only difference is a 29% difference in gradient time which corresponds to a 30% difference in dead volume (in translations flow x gradient time/dead volume should be kept constant). In this case co-elution of peaks 4 and 5 was observed.

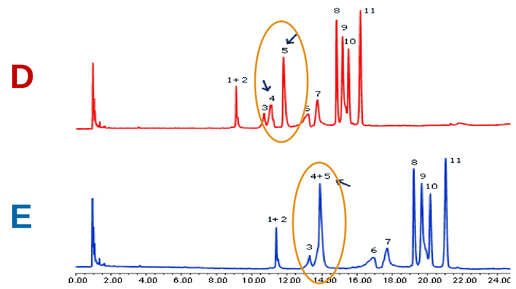


***Figure 4**: Chromatograms illustrating the impact of 29% error in dead volume on a gradient separation of a series of proteins.*

Since estimations of dead volume can be misleading, it is strongly recommended that V_M should be determined experimentally for accurate translations. This can be done by injecting a RP-LC dead time marker such as uracil or thiourea; or by using the first disturbance of the baseline after an injection of water to acetonitrile.

Factors in Instrument Design That Contribute to Inaccurate Translations

The effect of instrument differences between HPLC and UHPLC models have also been identified as possible sources of translation error.

Column thermostat design—experience shows deviations of 5ºC to be common. It is recommended that information provided in the system suitability section of the method is used to correct for the affect of temperature on selectivity.

Heat of friction—heat generated by depressurization of the column results in an axial temperature gradient and thus selectivity differences. These can be compensated for by increasing the temperature when translating from UHPLC to HPLC and decreased when translating from HPLC to UHPLC.

Pressure—changes in pressure must be compensated for by re-optimizing the method—making adjustments of gradient shape and temperature.

Other—a number of other factors may also result in unexpected selectivity differences when scaling chromatographic methods. For more details see [7].

A New Translation Tool

In order to assist the chromatographic community to successfully translate between HPLC and UHPLC methods, a new simple translation tool was developed. This tool is based on the principles described in this poster and permits scaling of gradient times, flow and injection volume as well as accounting for differences in V_D/V_m ratios between LC systems. In contrast to other applications it allows the use of experimental dead volumes. The translator is available free on the ACD/Labs website (Fig. 5).

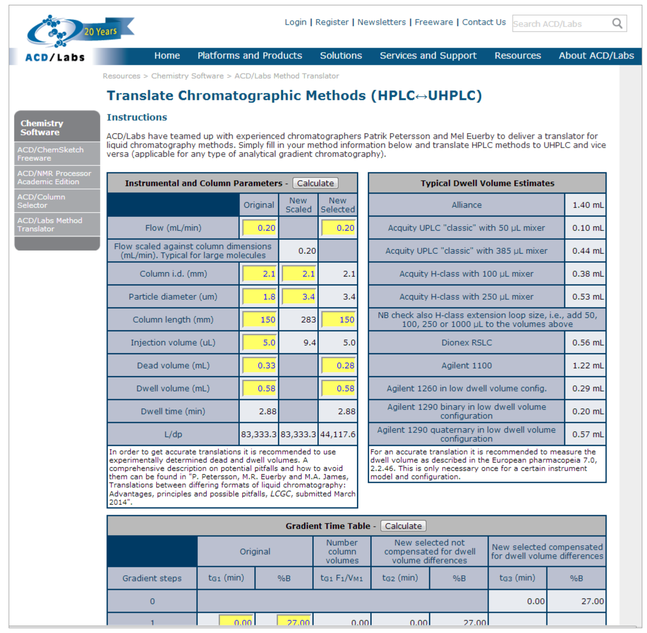


***Figure 5**: Free online method translator available on the ACD/Labs website (www.acdlabs.com/translatemymethod).*

The principles discussed in this poster have been successfully applied by the authors for routine scale up between analytical and semi preparative LC (150 x 30 mm x 5 µm) formats.

A more comprehensive translation tool that has been included in the commercially available software—ACD/Chrom Workbook. Contact ACD/Labs for a trial or demonstration. [8]

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