CHROM. 23 022

Short Communication

Compensated delay volume as an approach to improve the inter-laboratory transferability of gradient high-performance liquid chromatographic assays

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ABSTRACT

A novel method for minimizing differences in retention times between different gradient high-performance liquid chromatography (HPLC) systems is proposed. The gradient profile (*i.e.*, mobile phase strength *vs.* time) reaching the column is duplicated on each gradient system by adjusting an initial isocratic period in the assay to compensate for the measured delay volume of that system. An initial isocratic period must be included in the gradient profile to facilitate this approach. Suggested initial isocratic periods are 5 ml for standard HPLC systems and 0.5 ml for micro-bore systems.

One of the limiting factors in the inter-laboratory transferability of gradient high-performance liquid chromatographic (HPLC) assays results from the differences in the delay volumes associated with different instruments [1–5]. The delay volume is associated with that portion of the instrument through which a solvent gradient must be pumped prior to reaching the head of the column. Relatively simple methods are available for estimating the system delay volume [4,5].

In one approach to eliminating this source of difficulty, it has been suggested [1–4] that the sample be injected some time after starting the gradient to correct for the delay volume. This approach seems simple enough from the outset, but is mechanically flawed in that the autosampler generally provides the signals that start the gradient "clock" at the moment of injection. Therefore, the gradient cannot be started prior to making the injection. If a system is used where the pump controls the autosampler or the autosampler were to inject after a time delay, this approach would be successful.

The prediction of retention times for each solute in an assay on systems of different delay volume has also been suggested [5]. This approach uses a specialized computing package [6] and retention data obtained from at least two gradient runs.

An alternate technique using a "compensated delay volume" is suggested for overcoming this problem. In this strategy, an isocratic period is included at the beginning of each assay. For each target instrument, the length of the initial isocratic

time is adjusted such that the volume pumped during this period plus the system delay volume for that instrument is equal to the intended initial isocratic volume. In this manner, the gradient profile as function of time on the column may be identical for different instruments.

If the delay volume is divided by the flow-rate of the analysis to provide a delay time, t_d, then a gradient profile might be written as:

Time	%A	%B
0.0	95	5
$5.0 - t_d$	95	5
$45.0 - t_d$	5	95
etc.		

It should be noted that the initial isocratic period designed into an assay must be larger than the delay volume of the HPLC system or complete compensation of the delay volume will not be possible. Some older equipment and a few current autosamplers have large delay volumes. Based upon a survey of modern instrumentation in use in our labaratory, a value of 5 ml has been selected for the initial isocratic period for each assay when traditional columns are used. For equipment suitable for use in micro-bore separations, a smaller volume might be employed (e.g., 0.5 ml). In either case, the initial isocratic period should be designed into the assay during the development period for maximum effectiveness.

In some situations, the recommendation of an isocratic period at the start of the gradient separation may not be feasible. Assays can be envisioned in which a number of weakly retained solutes may require an immediate gradient ramp (i.e., no initial hold) for efficient chromatography and/or resolution. For those methods where an initial isocratic hold cannot be designed into the assay, the inter-laboratory transfer of the method may be more problematic.

An example of the utility of the "compensated delay volume" approach may be seen in Fig. 1. Nominally, the same sample and mobile phase was used for each
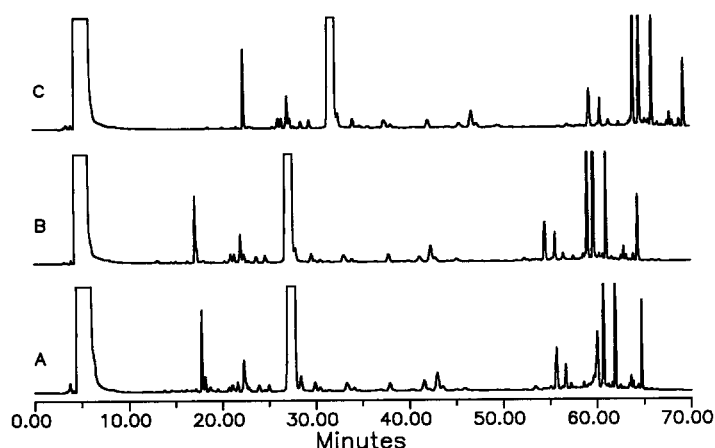

Fig. 1. Gradient chromatogram demonstrating effect of compensating for gradient delay volume. Please refer to the text for details.

chromatogram. Curve A represents the "development system" utilizing an HPLC with a 0.26-ml delay volume. As the assay flow-rate is 1 ml/min, the isocratic hold time was adjusted to 4.74 min for a total isocratic volume of 5 ml. Curve B represents a second instrument with a delay volume of about 4.5 ml and an adjusted initial isocratic period of 0.5 min. Note that the retention times of the components match well between these curves. Curve C shows the result of ignoring the 4.5-ml delay volume of the second instrument and programming a 4.74-min isocratic period for a total initial isocratic volume of over 9 ml. The difference in retention times between curves B and C along with the resultant uncertainty in identification is typical of inter-laboratory transfer of gradient methods when delay volumes are not considered.

The "compensated delay volume" strategy is a general approach which may be used for the effective transfer of gradient assays. There are no hardware or software requirements, but assays should be designed with an initial isocratic volume to allow for effective delay volume compensation.

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