

Liquid Chromatography Problem Solving and Troubleshooting

Question

My coworkers and I were discussing what is the best compound to use when measuring the void volume of a column. What is your recommendation?

Answer

The void volume of a liquid chromatographic system (V_0) corresponds to the volume that a totally unretained compound must traverse between the injector and the detector. It includes the intraparticulate volume (pores of the column packing) and the interstitial volume (the volume between column particles), as well as the volume of tubing and any other components between the injector and detector. The V_0 is a fundamental parameter for the liquid chromatographer. It is required to calculate the capacity factor (k'), which is used in a wide variety of other important chromatographic relationships. In addition, knowledge of the V_0 of a given system is required in the optimization of many chromatographic methods. Finally, if one is interested in determining thermodynamic evaluations of solute–solvent interactions, this requires knowledge of the V_0 . There is often much discussion regarding the most appropriate definition of the V_0 and how it should be measured.

Many analysts do not require a rigorous or elegant procedure to determine V_0 . They only desire a simple, rugged, and convenient way to obtain a reasonably good estimate of the V_0 of their chromatographic system. In a published study, several potential V_0 markers were studied including acetone, uracil, and deuterium oxide (D_2O) (1). The first two techniques used the UV detector, and the D_2O used the refractometer. The markers were investigated in the common high-performance liquid chromatography (HPLC) mobile phases of methanol–water, acetonitrile–water, and tetrahydrofuran–water mixtures. One of the major benefits of using uracil as a marker is that the observed value is fairly consistent as a function of concentration and can be used with the popular UV detector. Other potential markers exhibit a change in the observed value of the V_0 as the concentration of the solute changes.

Also investigated was the technique of using a homologous series that involves more rigorous experimentation as injections of the homologous series of compounds are required. The data manipulation includes linearizing the retention data with extrapolation of the retention volume to zero carbon number. This approach, however, resulted in a V_0 that was approximately 20% greater than that found using uracil as the marker (1), and because the technique of using a homologous series requires a significant amount of data manipulation, the report suggests that this technique is not a foolproof tool for the practicing chromatographer.

The chromatographer who requires a simple and reliable technique to measure the V_0 of the HPLC system will be well served by considering the use of uracil as the marker. This compound appears to be nonretained and exhibits very small change in the V_0 as a function of temperature and flow rate. However, it is conceivable that uracil might be retained on a strongly hydrophobic column. Therefore, when using uracil, it is advisable to confirm that it is not retained by injecting in a strong solvent as mobile phase and determining where it elutes.

In summary, uracil is readily available with a high level of purity, and it provides a single sharp peak with absorbance at 254 nm, making it a convenient marker. The compound of D_2O gives results similar to uracil, but requires the use of a refractometer that some users do not have in their laboratory.

References

1. B.A. Bidlingmeyer, F.V. Warren, A. Weston, C. Nugent, and P.M. Froehlich. Some practical considerations when developing the void volume in high-performance liquid chromatography. *J. Chromatogr. Sci.* **29**: 275–79 (1991).

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward it to: JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting*, P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

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