

Dwell volume: Misleading and mysterious

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Abstract

Minimization of pump dead volume below 0.1 ml has been essential to recent advances in the field of Ultra High Pressure LC. However, an accurate assessment of the pump dwell volume, which is essential step in the method transfer, is not a simple task. Different manufacturers measure dwell volume using different methodologies. As a result, the calculated values may vary and may even be misleading. Currently there is no consensus on the best methods to use. Therefore, a uniform protocol for pump volume measurement is essential. We have investigated pump dead volume using UV –based techniques (slope intercept and half-max signal) and compared this with absolute measurements, and with standard volume addition. We found that different approaches and conditions of measurement generate different results. Surprisingly, we found that the gradient delay volume is different from the physical volume stored inside of the pump (dwell or dead volume). The next paradoxical finding is that gradient delay volume is strongly dependent upon pump hardware and mixer design, rather than actual pump internal volume. In consequence, 2 identical internal volumes may result in different gradient delay volumes. Our next important finding was that the use of a linear gradient provides more accurate information compared to a step gradient for delay volume evaluation. On the other hand, we found that a step gradient is an excellent source of information about pump equilibration dynamics (we have termed this the dispersion factor), which can be an additional characteristic of pump performance.

Biography

Eduard Rogatsky completed his M.Sc in physical chemistry from Belarus State University, PhD in Bioanalytical chemistry from Bar-Ilan University (Israel) in 1999, and postdoctoral studies at Albert Einstein College of Medicine, NY. He joined the faculty there in 2001, and is currently a Senior Associate Scientist and Director of Mass Spectrometry in the Biomarker Analytical Resource Core Laboratory, Einstein-Montefiore Institute for Clinical and Translational Research, Bronx, NY, USA.