Beyond Column Chemistry: Exploring External Variables Affecting Peak Shape and Width in Separations

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ABSTRACT

Introduction: Analytical chemists are trained to closely examine the separation columns, their surface chemistry, and mobile phase interactions to achieve the highest resolution in the shortest possible time. However, several factors can affect the separation process outside the column, especially the peak shapes. Unfortunately, many researchers often overlook these peak-distorting factors and compromise their separation quality. In addition, extra-column effects such as connection tubings and detection parameters, data sampling rate, and response times of different embedded digital filters can affect peak shapes. Although response time and data sampling rate have been discussed for more than 50 years in the separations community, their impact on peak shapes remains a subject of substantial interest to researchers involved in developing signal processing methods and software for chromatography data acquisition.

Objective: This talk will explore the overall impact of extra-column effects. How do metal components distort peak shapes? What are the response times for HPLC-UV detection parameters, and what do they represent in signal processing? How many data points should we sample per peak? Addressing these questions and understanding the digital filters used in chromatographs can help mitigate peak shape distortions due to data acquisition software.

Methodology: This discussion will also focus on conceptualizing sampling rates, response times, and various denoising processes used in high-performance liquid chromatographs through simulated and real examples. These factors can become significant in low signal-to-noise ratio environments. Solutions to practical separation science problems will also be proposed.

Result & Conclusion: Symmetric peaks in any separation mode are highly desirable because they allow reliable integration compared to other asymmetric peak shapes/models. We will first present the results of peak shape distortion arising from the packed bed structure of stationary phases. The effects of metallic components of an HPLC affect biomolecular separations significantly. Similarly, different instruments and their data acquisition software involve "black box" digital processing methods, negatively affecting peak widths and shapes. These issues become increasingly significant with the availability of high-efficiency core-shell particles and better instruments with fluidic designs. Examples will be presented from the presenter's research and literature.

Keywords: Extracolumn Band Broadening, High-Efficiency Separations, Peak Shapes, Signal Processing.

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