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Note d'application

Influence of Sample Injection Parameters on the Performance of UltraPerformance Convergence Chromatography (UPC^2) Coupled with MS Detection

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the influence of sample injection parameters on detector response and peak width for the analysis of polar analytes by UltraPerformance Convergence Chromatography (UPC^2)-MS.

Benefits

Chromatographic peak width and detector response in UPC^2 analysis are greatly influenced by sample injection parameters, and therefore must be considered during method development.

Introduction

The optimization of method parameters is an important task when developing both qualitative and quantitative methods. Sensitivity, resolution, and run time must all be balanced depending on the goals of the analysis. The introduction of MS as a detection method can add further complexity and increase the time required to develop a robust and reliable method. In the work presented here, we evaluate the effect of sample injection volume and composition on chromatographic peak capacity (P_C) and MS detector response when coupled to UPC^2 for the quantitative analysis of a mix of polar analytes that included nucleosides, nucleobases, and caffeine.

Results and Discussion

For both quantitative and qualitative analysis the chromatographic peak width and thus peak capacity is an important parameter that must be considered. The peak capacity of a chromatographic separation is dependent on both the length of the gradient as well as the width of a chromatographic peak. Higher peak capacities are generated by narrow peak widths.

These narrow chromatographic peak widths result in increased analyte resolution from matrix and greater peak heights, enabling lower limits of quantification and the minimization of matrix effects in the MS detector.

The data from this study shows both the effect of sample injection composition as well as the injection volume on both the peak capacity of the chromatographic separation produced by the UPC^2, and the effect on the signal produced by the MS detector. Figure 1 illustrates the influence of the sample composition on separation peak capacity. The compositions of the sample injection solvent varied in the ratios of water and acetonitrile, ranging from 1:9 (water:acetonitrile) to 9:1 (acetonitrile:water). As can be observed in Figure 1, the average peak capacity of the separation was greatly reduced when the injection volume was increased. Values ranged from P_C=180 for a 1 µL injection to P_C=120 for a 5 µL injection. When low amounts of water were utilized, up to a ratio of 1:9 (water acetonitrile) the peak capacity for the separation was highest (P_C =180) for all analytes in the test mix. It was also noted that the lower water composition resulted in higher peak capacities across all of the injection volumes.

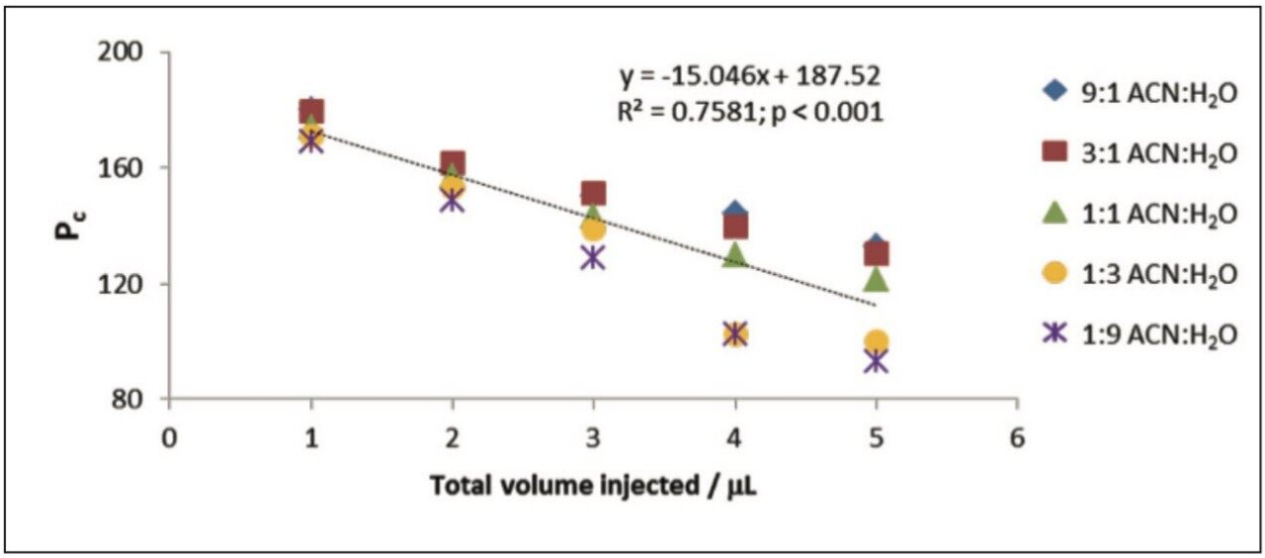

Figure 1. UPC^2 peak capacities for test mix in ACN:H_2O solutions plotted as a function of the volume injected.

Figure 2 illustrates the response of the MS detector as a function of peak capacity and injection volume. In Figure 2 we observe that the higher injection volumes in general lead to lower peak capacities, but higher MS responses. Conversely, lower injection volumes resulted in a smaller response from the MS detector, but higher peak capacities. Figure 2 further illustrates that for the test mix utilized here in a solvent composition of 9:1 (acetonitrile:water), the best compromise between peak capacity and response was observed with a 3 µL injection, which typically gave above average Pc and MS response. Therefore, the results from the data produced in this study indicate that low injection volumes, such as 1 µL should be recommended when resolution of analytes is important (e.g., for qualitative analysis), and injection volumes of 3 µL are recommended when high response is required (e.g., for low limits of detection for quantitative analysis), as this represents the best compromise of highest MS response and peak capacity.

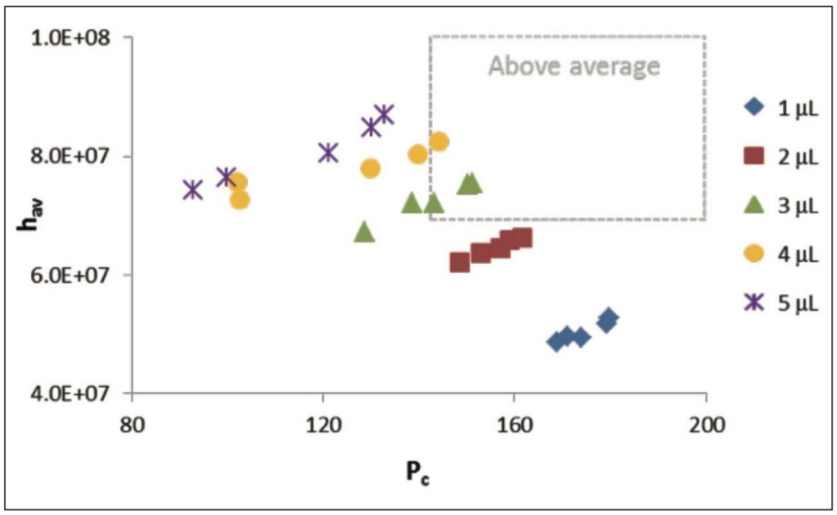

Figure 2. Plot of MS response (hav) versus UPC^2 PC. The region of the plot in which hav and PC values are above average is highlighted.

Conclusion

This study presented the influence of sample injection volume and composition on both the response produced from a MS detector, and on the peak capacity produced by the chromatographic separation. Higher concentrations of water in the sample solvent and higher injection volumes resulted in lower chromatographic peak capacities produced by the separation, while producing higher MS response. Therefore, depending on the type of analysis being carried out, i.e., qualitative or quantitative, one must balance the sample injection parameters to obtain the aim of the analysis.

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