

Application of Weak Reversed-Phase Retention on Silica Column to Remove Matrix Suppression in LC-MS/MS

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INTRODUCTION

The retention mechanisms of silica column include hydrophilic interaction (HILIC), cation exchange with negatively charged silanol groups, and hydrophobic (or reversed-phased, RP) interaction with the siloxane bridges. The reversed-phase retention on silica column is rather weak compared to the one on C18 column. In this case study, we will describe a unique approach using this weak reversed-phase interaction to remove matrix suppression, which coeluted with analyte on both C8 column and silica column in HILIC conditions.

METHODS

The human plasma samples were extracted by Oasis™ HLB SPE columns. Matrix effect was observed first from variable internal standard responses on a C8 LC-MS/MS. A post-column infusion of analyte and internal standard solution was used to confirm and locate the matrix suppression from blank extract. The analytical columns used to optimize the separation or removal of matrix suppression from analyte and internal standard included Waters Atlantis™ HILIC Silica 3.0 x 50 mm, YMC Basic C8 2.0 x 50, and Merck Chromolith Speed Rod RP-18e C18 4.6 x 50 mm.

RESULTS

The initial results from the post-column infusion tests showed matrix suppression coeluted with analyte and IS under different gradients for HILIC, C8 and C18 columns. It indicated that the matrix effects came from components with very similar polarities as analyte and IS. There was no retention time change with different pH values in mobile phase on silica column. Therefore, ion exchange mechanism did not apply in this case.

We observed reversed-phase retention on silica column for analyte and IS with bulky hydrophobic group when organic content of mobile phase lowered to 15%. Under this condition, all the matrix effect components could not retain on the silica column and eluted out in the solvent front. The data presented will highlight the robustness and speed of this unique application of silica column for bioanalytical LC-MS/MS work.

Figure 1: IS Area Plots

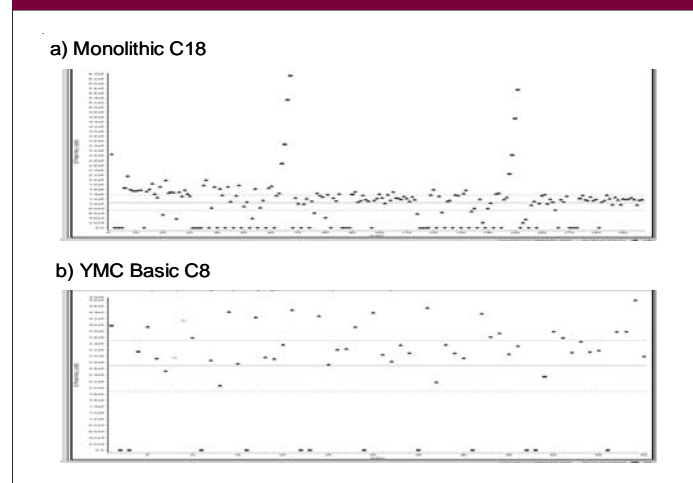


Figure 4: Matrix Effect Chromatogram on Si Column in HILIC Mode

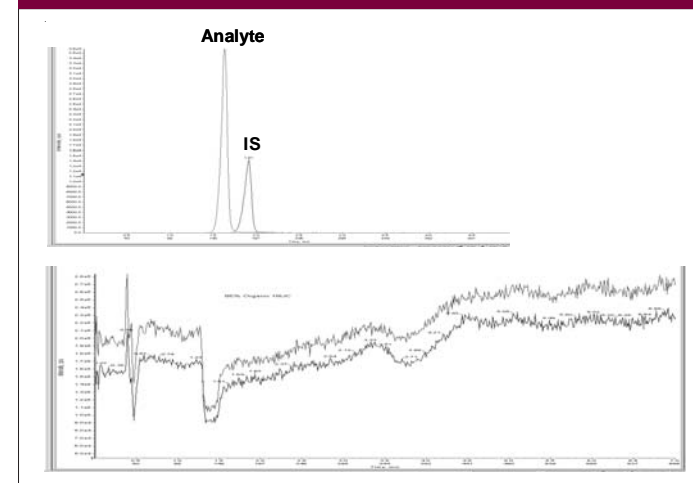


Figure 7: Peak Shape and Rt on Si Column with Various Organic % B

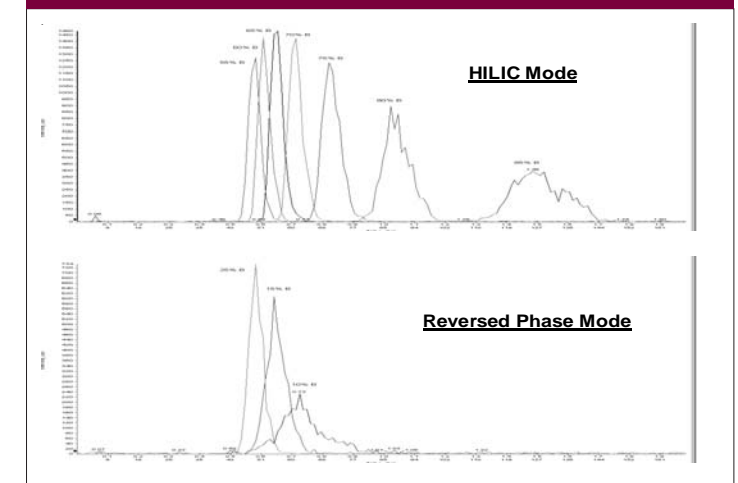


Figure 2: Post-Column Infusion for Matrix Effect Detection

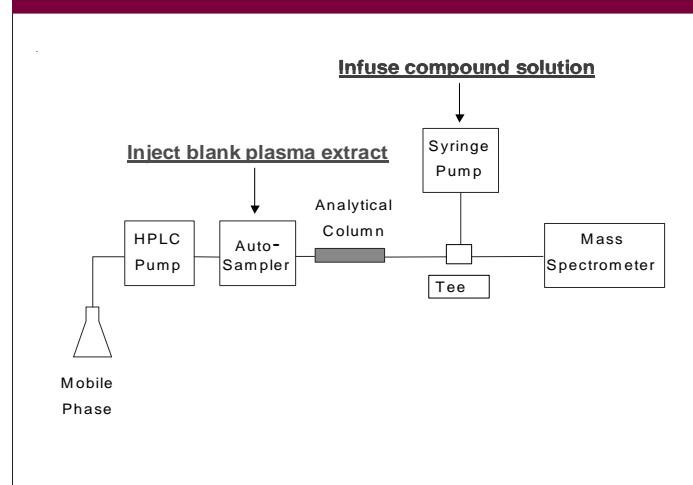


Figure 5: Matrix Effect Chromatogram on Si Column in RP Mode

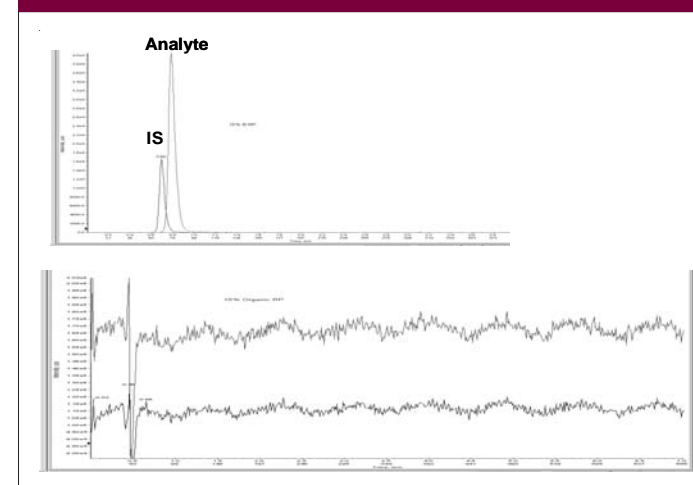


Figure 8: Peak Shape and Rt on Si Column with Various Organic % B

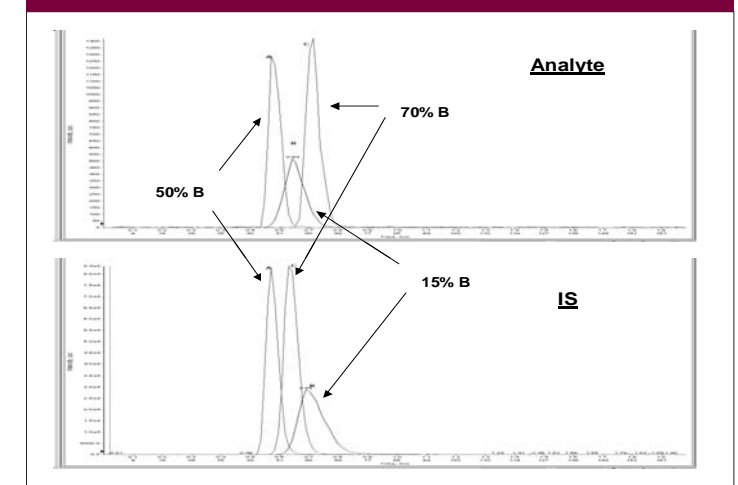


Figure 3: Matrix Effect Chromatogram on Reversed-Phase Column

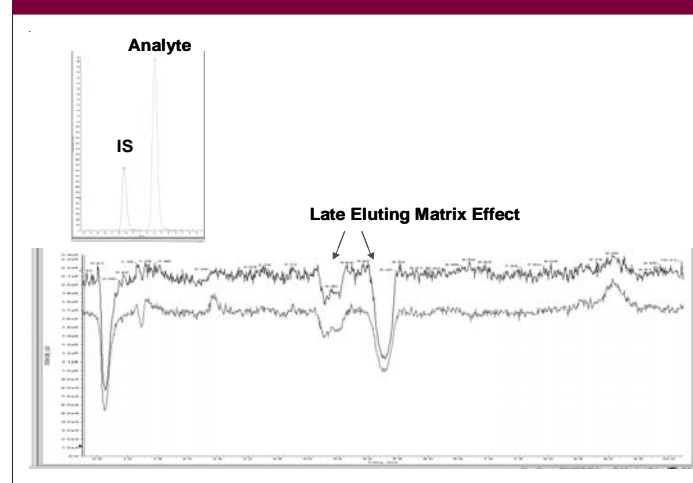


Figure 6: IS Area Plots Using Si Column in Reversed Phase Mode

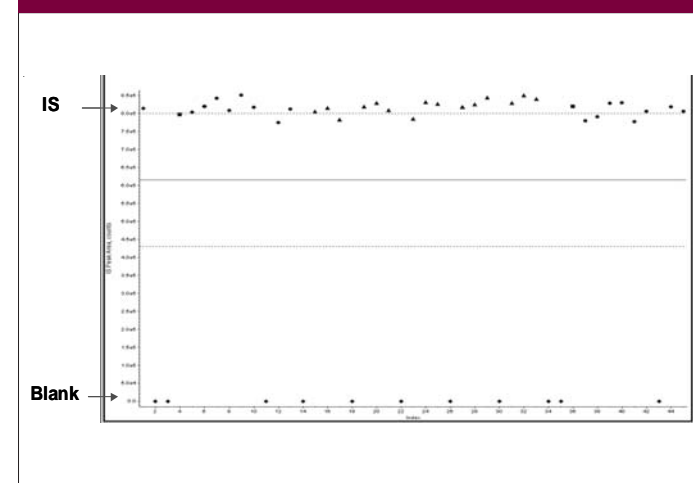


Figure 9: Retention Time on Si Column with Various Organic %

