

Use of Conventional LC Instrumentation in a Comparison Study of Semi-Porous and Porous Particles for Fast LC-MS-MS Analysis

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Fast bioanalysis requires using short columns packed with high efficiency particles. HPLC columns packed with fused core particles or porous particles in the sub-2 μm range can enhance both resolution and speed by producing either higher efficiency (N) for the same column length or equivalent efficiency with a shorter column length. The use of ultra-high performance liquid chromatography (UHPLC) comes at a price of higher pressure when sub-2 μm particles are employed. As an alternative, the fused silica particle has become accepted because it's equivalent in performance to particles in the sub-2 μm range. These fused core columns employ conventional 2 μm frits and operate ruggedly at much lower pressures that are within the operating limits of conventional HPLC instruments.

A 50 mm column containing porous particles was initially used to run this analysis. The goal was to increase speed and throughput so the 50 mm column was replaced with a 20 or 30 mm column containing fused core particles. Gradient slopes were adjusted to accommodate the column lengths. Drug containing samples were extracted from plasma based on a published method and analyzed by LC-MS-MS. Calibration curves and quality control samples were run and peak width comparisons made between the columns studied to determine efficiencies. The entire analysis was carried out on a standard LC instrument interfaced to MS-MS.

The goal of this work was to demonstrate the ease of converting traditional multi-minute assays to one-minute assays using a 20 or 30 mm fused core column on a standard LC-MS instrument. In addition to this faster analysis, chromatographic resolution and peak shape were maintained over the conversion. Better efficiency was obtained using the fused core columns, even though the analysis was run at higher flow rates. Good linearity was also seen using these shorter columns. System backpressures were maintained at less than 240 bar for these analyses indicating that standard LC pumps can be routinely used with columns containing fused core particles.