

HPLC-UHPLC Hybrid 2D Platform for LC/MS Analysis of Biological Samples. Back to the future.

Eduard Rogatsky and Daniel T. Stein

Biomarker Analytical Resource Core,

Einstein-Montefiore Institute for Clinical and Translational Research.

Albert Einstein College of Medicine of Yeshiva University, Bronx, NY 10461

Abstract

Modern UHPLC systems have important advantages over previous generations of HPLC systems. These are (a) higher pressure limits, and (b) considerably smaller delay and post column volumes, which are essential for the development of fast gradient applications. Fast gradient applications by UHPLC/UV systems are easily adopted in QC/QA applications. However, the analysis of complex samples was found to be more challenging and costly, if UHPLC technology was used. There are several reasons for this including;

- Clinical sample analysis usually utilizes a large volume injection of a diluted sample, which is suboptimal for a UHPLC autosampler.
- Extracted samples obtained after sample preparation steps, drying and reconstitution may have small particulates that shorten UHPLC column lifetimes.

We developed a cost-efficient hybrid LC platform that's ideal for biological sample analysis by LC/MS. This platform is based on Agilent 1100 and Agilent 1290 series LC devices. The first dimension of the analytical system used a standard pressure range (400 bar max) binary HPLC pump, where the sample was injected using a standard pressure (not UHPLC) autosampler into a pre-analytical column. The sample was then desalted, partially purified, and concentrated using the pre-analytical column.

The fraction containing analytes of interest was transferred through a UHPLC valve to a fused core column. Once the analytes were transferred, a fast gradient was performed by using a UHPLC 1290 pump. The addition of a UHPLC valve and pump to a standard 1100 series LC system (autosampler, pump and column compartment) greatly extends operational flexibility including column selection, while standard LC – which is already available in the lab, performs the initial steps of sample loading and clean-up.

We successfully used this platform for LC/MS analysis. Instead of retiring an entire functioning Agilent 1100 LC system, we just added one UHPLC pump to achieve much greater

overall performance, functionality and lower cost, compared to a single pump UHPLC system purchase.