A high throughput mass spectrometry plate reader: Echo® MS System

An acoustic droplet ejection to an open-port sampling interface

Label-free Liquid Chromatography/Mass Spectrometry (LC/MS) based screening technology is routinely used in pharmaceutical industries for hit discovery and various Absorption, Distribution, Metabolism, and Excretion (ADME) profiling applications. Although the current analysis speed of <30 seconds per sample is quite promising, it still cannot match the throughput provided by plate-reader based High Throughput Screening (HTS) platforms. Acoustic droplet ejection (ADE) is a droplet transfer technology capable of high speed, reproducibility and absolute accuracy. In this work, we successfully couple ADE and the standard Electrospray Ionization (ESI) ion source of a mass spectrometer with the open-port probe (OPP) sampling interface. Screening speeds as fast as 0.4 seconds-per-sample (2.5 Hz) are demonstrated with high sensitivity, high reproducibility, good quantitation capability, low matrix ion suppression effect, and broad compound coverage from small molecules to peptides and antibodies.

ADE is performed using piezoelectric transducer technology, which is in routine use e.g. for acoustic sampling. The OPI is positioned above the plate so that ejection of 2.5-nL sized droplets are quantitatively captured by open sampling end of the OPI and rapidly transferred to standard ESI source. Fast moving 384 or 1536 micro well plates provide extremely speedy well-to-well movement to ensure highest acquisition speed and, thus, highest throughput.

We analyzed various parameters relevant to the performance of the integrated ADE-OPI-ESI-MS system. The “classic” ESI ion source for MS analysis yields a perfectly Gaussian-shaped signal peak with baseline width of 0.8-1.5 seconds. High sensitivity (attomole loading for small molecules, or sub-femtomole for intact antibody) and high reproducibility (Coefficient of Variation CV<8%) was demonstrated for this platform, showing good linearity over at least three orders of magnitude even without internal standard. The 2.5-nL sized droplet was diluted within the sampling end of the OPI by several hundred fold, thus very low matrix effects were observed even with incubation buffers containing detergents. The continuous flow of the carrier solvent maintains ionization stability and also actively cleans the entire flow system, thus eliminating carry over. In addition, the “classic” ESI ion source used in this setup enables detection of a broad range of analytes including small drug molecules, peptides (MW>1500 Da) and large proteins (up to 150 kDa). HTS applications were successfully demonstrated with a robust Z’>0.7. The screening throughput can be further improved by multiplexing. 0.4 seconds-per-well sampling rates were demonstrated by judicious arrangement of samples in consecutive wells, and this is limited only by the speed of mechanical stage movement.