Improving Data Quality via Ratiometric Assays Using Microparallel Liquid Chromatography

Nanostream, Inc.

The Nanostream® Veloce® micro parallel liquid chromatography (μPLC) system (Nanostream, Inc., Pasadena, CA) and ratiometric assay analysis software were used to analyze and process data for a screen of approximately 6000 compounds. The results of this study illustrate the key benefits of microparallel liquid chromatography as a powerful new screening tool.

The performance of modern high-throughput screening (HTS) tools such as imagers and plate readers serves as the benchmark for drug discovery efforts industry-wide. These tools are typically fast and sensitive but not very selective. Chromatographic techniques such as HPLC are extremely selective but not suitable for high-throughput drug discovery applications because these techniques are inherently low throughput, require large volumes of samples, and present issues related to solvent consumption and disposal. The Veloce μPLC system, used in conjunction with 24-column Brio® cartridges, combines the selectivity of HPLC with qualities that are essential for success in drug discovery applications, e.g., higher throughput. Further, the Veloce advanced analysis module for ratiometric assays provides a rapid readout of the ratio between the substrate and product, which reduces assay variability

Experimental Conditions

The enzymatic reactions were carried out in 384-well plates with a total assay volume of 60 μL in each well. Each well contained 125 nM methionyl peptidase, 50 μM MASW substrate, 8.3 μg/mL of target compound, and 50 mM MOPS, pH 7.2 containing 100 μM $CoCl_2$. The reactions were incubated for 1 h and were stopped with 25 mM EDTA. 5-μL aliquots of sample were then injected via an integrated multichannel autosampler onto the Brio cartridge (Model 4208004), which consisted of 24 parallel columns (80 mm × 0.5 mm) containing 7-μm modified C18 stationary phase. Both product and substrate peaks were monitored by fluorescence detection (λ_{ex} = 254 nm and λ_{em} = 330 nm). The mobile phase flow rate was 300 μL/min per cartridge (or 12.5 μL/min per channel). Mobile phase A consisted of 0.1% TFA in deionized water while mobile phase B consisted of 0.1% TFA in acetonitrile. The substrate and product peaks were separated under a gradient profile beginning with 15% B ramping to 25% B over 7 min, followed by a column wash (100% B) for 2 min, and returning to 15% B for a 3-min equilibration prior to the next injection.

Results

The ratiometric assay analysis module was used to automate analysis of over 6000 chromatograms and to output the data as %inhibition in a tabulated form (Figure 1). The use of ratiometric values used to calculate %inhibition yielded an improvement in assay performance, as defined by Z'-factor (compare Z' = 0.86 for the μPLC system compared to 0.76 from an equivalent assay performed on a conventional plate reader). This improvement translated to a lower 3σ cut-off above background, allowing data mining at a 2 × lower %inhibition compared to that of the plate reader. Further, since compound interference was eliminated, false positives and false negatives were significantly reduced.

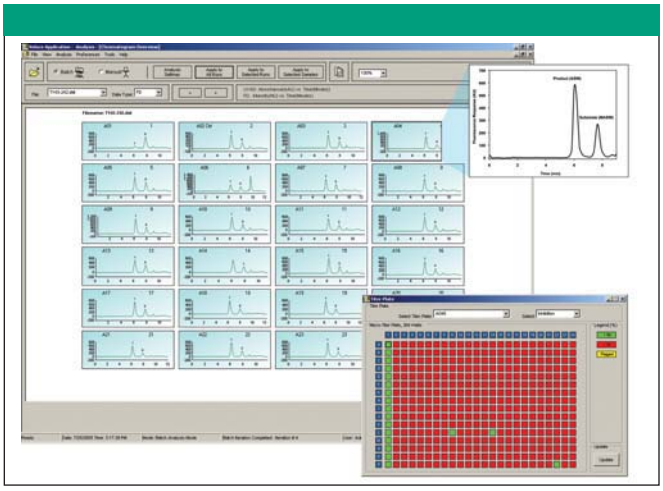

Figure 1: Thumbnail of ratiometric assay analysis module with overlay of titer plate. The advanced analysis module calculates the ratio of peak areas for both the product and substrate peaks using a predefined time window. A representative chromatogram depicting the separation of the product and substrate at 7.2% compound inhibition is highlighted.

Conclusions

Microparallel liquid chromatography was used in a campaign to screen a total of 17 384-well plates. All of the chromatograms were easily processed, and results were tabulated in a format familiar to HTS scientists by using the Veloce system ratiometric assay analysis module. By employing a separation-based assay to obtain a substrate-to-product ratio, compound interferences were significantly reduced.

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