



High Throughput Determination of Compound Solubility with Micro Parallel Liquid Chromatography

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High throughput determination of compound solubility can be achieved with the Nanostream CL micro parallel liquid chromatography (μ PLC) system (Nanostream Inc., Pasadena, CA) that offers increased sample analysis capacity, fast data analysis, and reduced sample consumption, solvent usage, and waste generation.

Aqueous solubility constitutes a critical parameter that needs to be determined during drug lead optimization (1). Traditional methods for solubility determination typically consume large amounts of compound, and conventional methodologies and analytical techniques are inherently low throughput. Micro parallel liquid chromatography (μ PLC) offers a high-throughput, low volume analytical approach for the routine assessment of compound solubility.

Experimental Conditions

The μ PLC system employed in these determinations (Nanostream CL System, Nanostream Inc.) is equipped with 24 parallel columns for liquid chromatography, each with its own sample introduction port and exit port for connection to UV absorbance detectors (2). Flow from a binary solvent delivery system is divided evenly across 24 channels so that 1/24th of the programmed pump flow rate passes through each column. Samples are introduced to the cartridge by a multi-channel autosampler, which is configured to sample from SBS-standard 384-well plates.

Sets of calibration standards were prepared for 24 compounds at 4 different concentrations. External standard calibration curves were determined for each compound (peak area versus concentration). Compound solubility values were evaluated from interpolation of the corresponding compound peak area obtained from a solution prepared with appropriate buffer (pH = 7.4 for this study) within the corresponding external standard calibration curve. Accurate and reproducible control of the depth of the autosampler needles permitted the sampling of the supernatant solution without perturbing the precipitate, thus avoiding the need for sample filtration. This approach significantly reduces sample preparation requirements as well as consumable expenses.

Results

Sample chromatograms obtained simultaneously for the standards (500 μ M) of the 24 compounds investigated are shown in Figure 1. Data analysis was performed using the advanced analysis software to automate the analysis of samples and generation

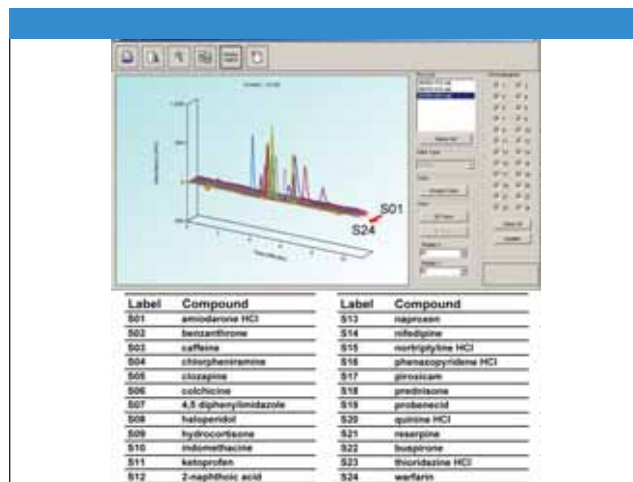


Figure 1: Sample chromatograms obtained simultaneously for the standards (500 μ M) of the 24 compounds investigated.

of calibration curves. Linear regressions were evaluated for all standard curves with R^2 values between 0.983 and 1.0. Aqueous solubility values for the 24 samples compared favorably with results obtained by other methods.

Conclusions

These results demonstrate the utility of the Nanostream CL System for high-throughput determination of compound solubility. The approach allowed for the generation of calibration curves for 24 compounds (8 replicates at each concentration), replicate solubility measurements (4 replicates), and automated data analysis in less than 3 h (not including incubation time). Alternative configurations allow for the analysis of 96 samples (4 replicates) or 192 samples (duplicates) in a proportional amount of time. Minimal sample quantities (5 μ L of 10 mM solutions in DMSO for this study) were required for all these determinations. Overall the use of this system significantly increased sample analysis capacity while reducing sample consumption, solvent usage, and waste generation.

References

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