



# Micro Parallel LC with Time-Triggered, Parallel Fraction Collection Accelerates Sample Preparation for MS Assays in Drug Discovery and Development

Nanostream, Inc.

**24 fractionated samples were simultaneously collected at user-specified retention times using the Nanostream® Veloce™ micro parallel liquid chromatography ( $\mu$ PLC) system (Nanostream, Inc., Pasadena, California). Off-line detection by MS permitted fast, serial quantitation of analyte concentrations.**

The Veloce  $\mu$ PLC system, used in conjunction with 24-column Brio™ cartridges, increases LC throughput for discovery analytical applications and separation-based assays. The addition of a time-triggered, parallel fraction collector facilitates the use of the Veloce system as a front-end sample-preparation system for drug metabolism, pharmacokinetic assays, and other bioanalytical applications. The fraction collector outputs collected fractions to SBS-standard plates to permit subsequent detection by MS, MS-MS, or any other desired mode of detection. A schematic of this workflow is depicted in Figure 1. Designed to permit unattended operation, the fraction collector incorporates an integrated plate changing system with a dedicated rinse station and the capacity to transfer up to 70 plates.

## Experimental Conditions

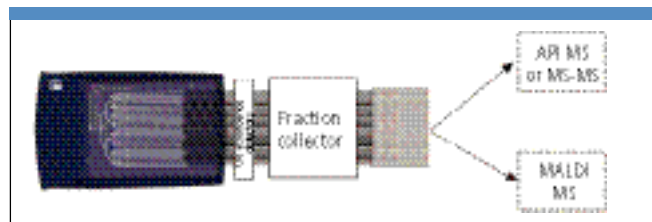
A series of 4  $\mu$ L dextrorphan injections at increasing concentrations (3.87  $\mu$ M to 3870  $\mu$ M) were injected onto a Brio cartridge (Model 4208002) at a 12.5  $\mu$ L/min flow rate per channel and collected as 4-min fractions onto a 384-well micro-titer plate. The fraction solvent was evaporated prior to reconstitution of each fraction in 30  $\mu$ L of 50:50 Acetonitrile:Water with 0.1% Formic acid. A 22- $\mu$ L loop injection (20  $\mu$ L loop) of each fraction was performed with a Thermo Electron Surveyor microwell autosampler into a Thermo Electron LCQ Advantage Max for mass spectrometric analysis.

## Results

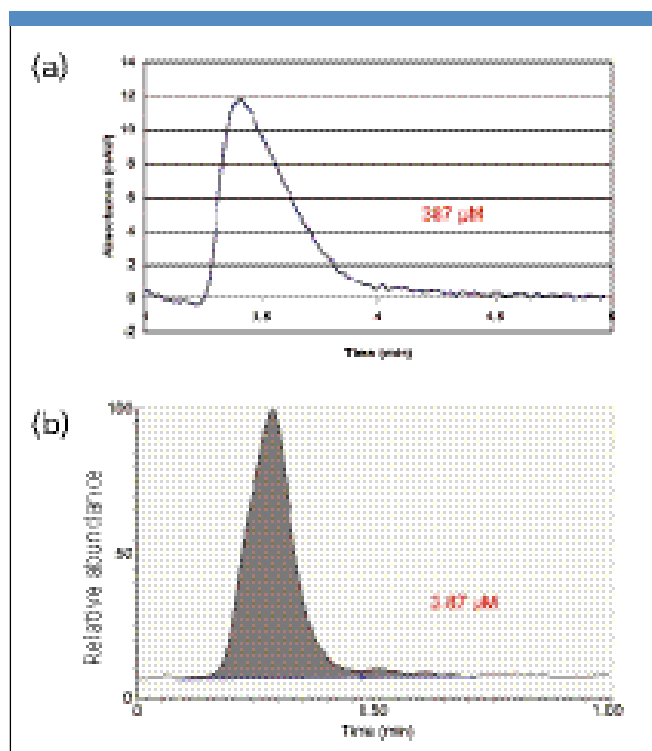
Figure 2 compares results for UV absorbance and MS detection for the range of concentrations analyzed. Micro parallel liquid chromatography with time-triggered fraction collection followed by MS was used to improve sensitivity by two orders of magnitude.

## Conclusions

Micro parallel liquid chromatography with time-triggered fraction collection can be used to overcome the LC bottleneck in routine MS screening. By decoupling the chromatographic separation from MS detection, analytical throughput can be conveniently increased for existing MS instrumentation.



**Figure 1:** Schematic representation of micro parallel LC analysis on a 24-column Brio cartridge (left) followed by collection of fractions into wells of SBS-standard microwell plate. Fractionated samples can be subsequently analyzed by numerous detection methods, depending on the specific requirements of each assay.



**Figure 2:** (a) The sample with the lowest concentration of dextrorphan (387  $\mu$ M) was detected by UV absorbance. (b) The sample with the lowest concentration of dextrorphan (3.87  $\mu$ M) was fractionated using the Veloce  $\mu$ PLC system with a time-triggered fraction collector and subsequently detected by MS using loop injection. This chromatogram demonstrates that sensitivity is improved by at least two orders of magnitude with MS detection.

Nanostream, Inc.

580 Sierra Madre Villa Avenue, Pasadena, CA 91107

tel. (626) 351-8200, fax (626) 351-8201

www.nanostream.com