New Compact Sampling and Sample Preparation Technologies for Portable Capillary Liquid Chromatography to Meet Today's Evolving Needs

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Abstract

High performance liquid chromatography (HPLC) is one of the most used analytical techniques in the world. Traditional analytical scale instrumentation consumes ~50 L of solvent per year, with a considerable benchtop footprint. By comparison, compact, capillary scale instrumentation offers comparable chromatography while consuming only ~100 mL of solvent per year and occupying roughly 12% of the same benchtop footprint. Additionally, a compact capillary scale automated sample preprocessing system that allows for process monitoring has been developed. The reduced solvent consumption and benchtop footprint make compact capillary instrumentation ideal for integration into space- and sample-limited workflows. This work will demonstrate the use of a sample processing system for online reaction monitoring. The low sample consumption makes capillary scale a desirable technique for biopharmaceutical analysis. A demonstration of the analysis of several critical quality attributes (CQAs) on the capillary scale will be shown. These advances in compact capillary scale instrumentation lay the foundation for greener, and more cost-effective chromatographic analysis.

Compact Sampling Module

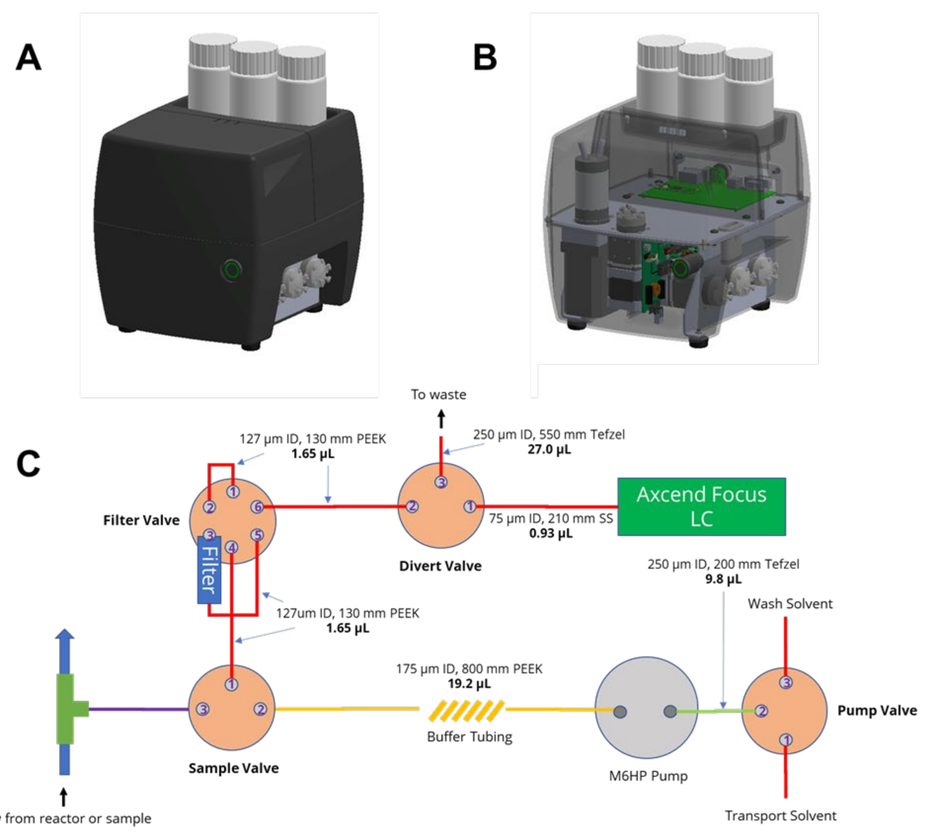

Figure 1. Drawings of the (A) external and (B) internal compact sampling module (CSM); and (C) general schematic fluidic diagram of the CSM.

Online Reaction Monitoring

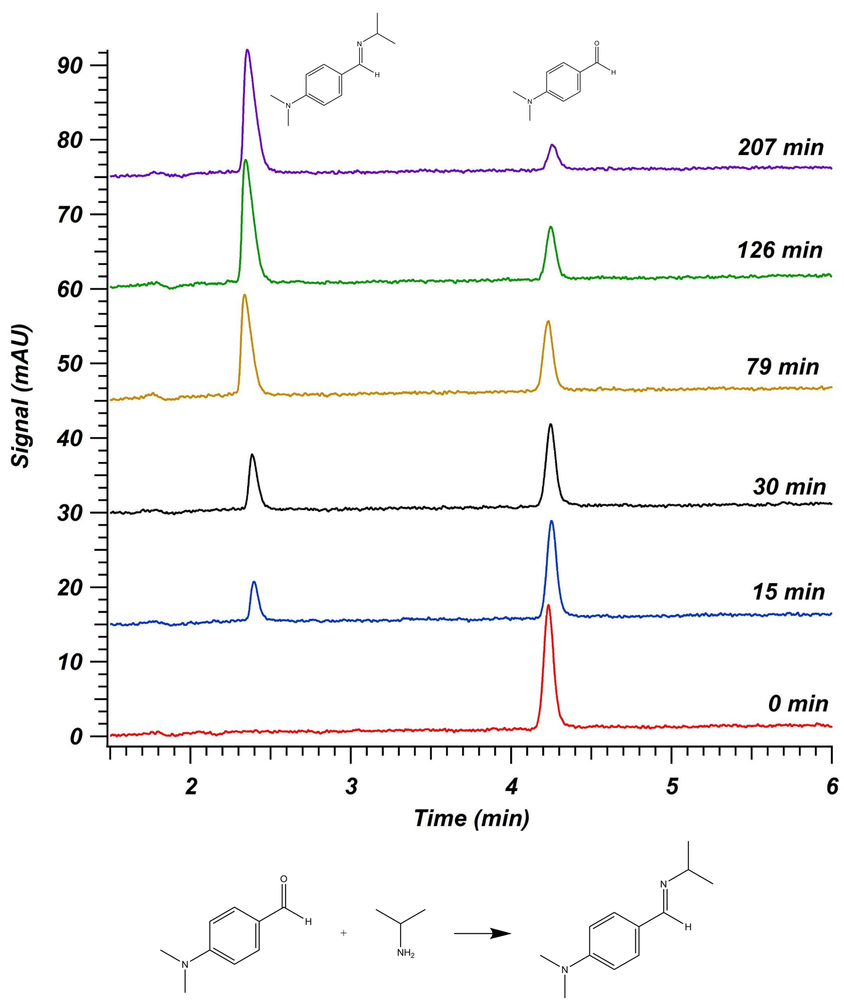

Figure 2. Series of chromatograms obtained during an imine condensation reaction between isopropylamine and 4-dimethylaminobenzaldehyde with samples taken every ~15 min over the course of 3.5 h. Less than 1 mL of waste was generated over the entire analysis (combined sample and mobile phase).

Repeatable Injections

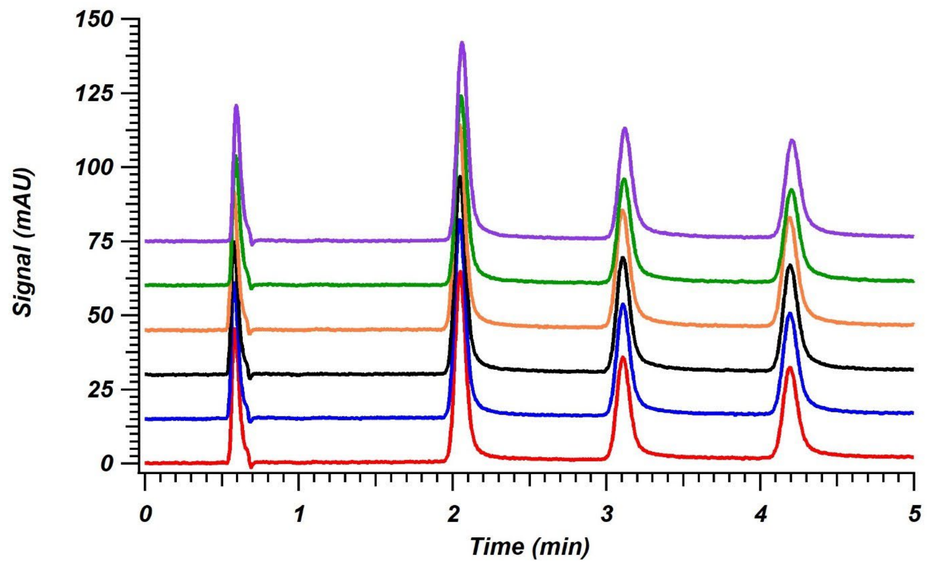

Figure 3: Repeatability results of six replicate injections utilizing the new Axcend InFocus sampling module for sample processing and loading, and the Axcend Focus capillary LC with UV detection at 254 nm. Peak identities in order of elution are thiourea, acetophenone, propiophenone, and butyrophenone. The percent relative standard deviation (RSD%) was approximately 2.4% across the four analytes.

Biopharmaceutical Analysis

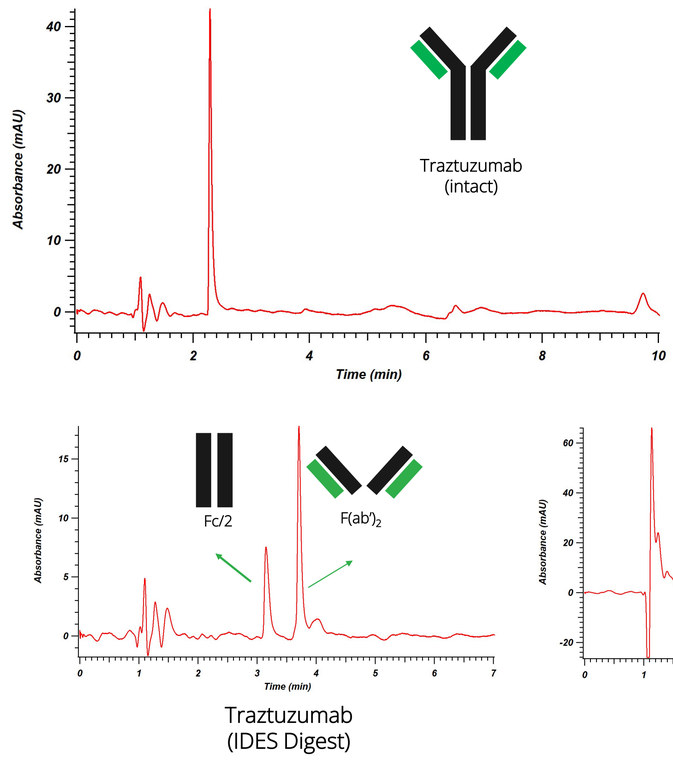

Figure 4: Separation of intact traztuzumab (1 mg/mL in water) using a Halo 1000 Å Diphenyl 0.2 x 100 mm column with 2.7 µm particles. The gradient was run at 3.1 µL/min going from 25-80% ACN over 10 min. The column was kept at 70˚C with detection being performed at 275 nm.

Figure 5: Separations of traztuzumab after IdeS digestion (left) and reduction (right) is shown. These common post translational modifications are used to verify fragment masses as an additional CQA.

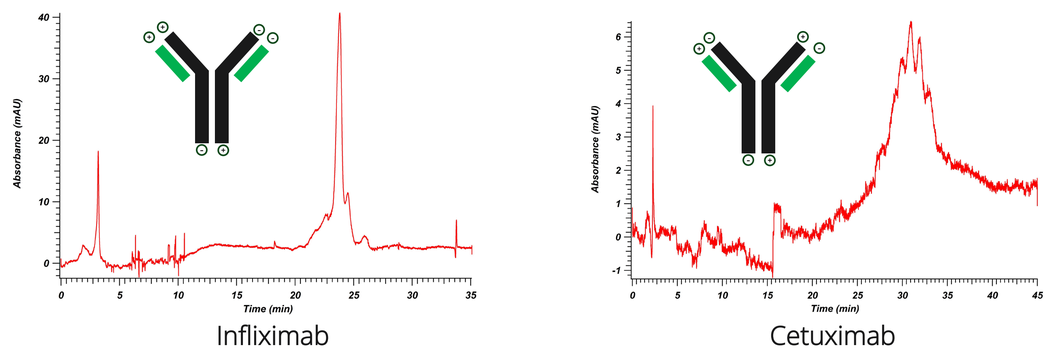

Figure 5: Separations using weak cation exchange to determine the charge variance (another CQA) of infliximab and cetuximab. Separations were performed using a PolyLC PolyCat A 1000Å 0.3 x 150 mm column with 3 µm particles.

Conclusions

Compact capillary scale LC is an ideal platform for both space- and sample-limited environments. Recent developments in small footprint sample handling systems allow for automated sample monitoring that can be easily integrated into existing workflows where space may be limited. Here an imine condensation was monitored every ~15 min over the course of over 3 h using less than 1 mL of total solvent. Additionally, the repeatability of this system was demonstrated, with RSD values for peak areas as low as 2.4%. Furthermore, due to low sample consumption, this system is an ideal candidate for monitoring various large molecule biopharmaceuticals, which require the validation of various CQAs. Demonstrations of intact and fragment analysis using RP as well as charge variance analysis using WCX were shown. These advancements enable automated, low volume monitoring of sample or reaction vessels using compact capillary scale liquid chromatography.

Acknowledgements

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