Exploring Biopharmaceutical Analysis with Compact Capillary Liquid Chromatography Instrumentation

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A recent trend in the design of liquid chromatography (LC) instrumentation is the move towards miniaturized and portable systems. These smaller platforms provide wider flexibility in operation, with the opportunity for conducting analysis directly at the point of sample collection rather than transporting the sample to a centralized laboratory facility. For the manufacturing of pharmaceutical and biopharmaceutical products, these platforms can be implemented for process monitoring and product characterization directly in manufacturing environments. This article describes a portable, miniaturized LC instrument coupled to a mass spectrometer (MS) for characterization of a biopharmaceutical monoclonal antibody (mAb).

Liquid chromatography (LC) continues to be a crucial technique for chemical analysis. The capability to effectively separate and quantitate a broad range of analytes in complex mixtures plays a critical role in the characterization of samples across a broad range of application, including pharmaceutical, environmental, clinical, forensic, biomedical, and industrial/manufacturing. Despite the importance across many areas of laboratory testing over the past six decades in which LC instruments have been commercially available, their general design has remained relatively constant. Although many improvements have been made to instrument components to increase their reliability, robustness, reproducibility, lifetime, and operating range during that time, the standard laboratory benchtop LC setup has seen few significant changes. In some ways, this enduring format speaks to the high confidence that many users have in the operation of modern LC instruments, typically abiding by the old adage "if it's not broke, don't fix it". However, in some cases, thinking about new approaches to LC technology can lead to significant changes in the capabilities and options available to analysts. Portable instrumentation provides the opportunity to perform analyses outside of laboratory settings and directly at the point-of-need—a capability that has seen growth for both spectroscopic (1) and mass spectrometric (MS) (2) analysis. Even in more traditional testing environments, the flexibility that a compact instrument provides in terms of reduced footprint and maneuverability can help enhance existing workflows. Based on these advantages, there has been a growing trend in the development of compact and portable LC instrumentation over the past decade (3).

Several properties have been used to define portable LC technology, including size, weight, power source and consumption, ease of operation, and waste generation (4). Some of these factors, including reduced weight and decreased mobile phase waste generation, suggest the adoption of capillary-scale LC columns for compact and portable instruments. Operating at flow rates that are 100–1000 times lower than typical analytical-scale LC methods minimizes the amount of mobile phase that must be carried with the instrument and the waste that is generated during analysis. Work towards achieving a completely portable LC instrument over the past decade

has focused on the development of high-pressure capillary-scale pumps (5,6) and detectors (7,8). The combination of these components into an integrated, portable instrument has resulted in a commercialized LC platform (9).

In the literature, the use of this instrument has been reported for the analysis of cannabinoids (10), biocides in wastewater (11), scopolamine analysis in beverages (12), and online monitoring of small volume synthetic reactions (13). In addition, multiple pharmaceutical companies have tested its use for various needs in their industry as part of an Enabling Technologies Consortium project (14).

This article describes the practical benefits of a miniaturized, portable capillary-scale LC system for the characterization of a biopharmaceutical monoclonal antibody (mAb) sample.

Characterizing Biopharmaceutical Antibodies with Compact Capillary LC

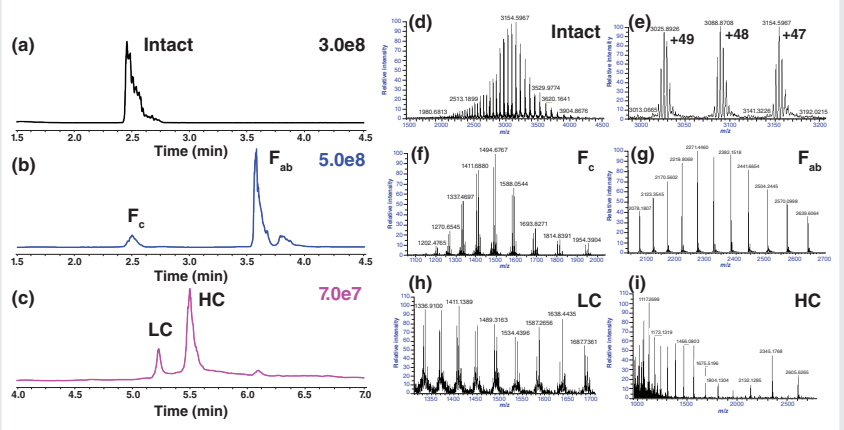
An increasingly important use of LC-based analysis is the characterization of therapeutic mAbs in the biopharmaceutical industry. A variety of critical quality attributes (CQAs) of mAbs can be measured as part of this characterization using LC coupled to MS. Recently, we explored the development of greener characterization techniques by translating various LC–MS mAb methods from 2.1 mm internal diameter (i.d.) columns to 1.5 mm i.d. columns (15). In some protein analysis methods, capillary-scale LC columns are adopted as a result of low quantities of available sample. Although this is typically not the case for routine mAb characterization in the biopharmaceutical industry, scaling down to smaller column diameters can significantly reduce the mobile phase consumption and make these methods much greener.

Although the methods employed for mAb analysis explored here are typical for LC analysis of mAbs in general, some of the new capillary LC system capabilities are useful for biomacromolecule analysis that differ from typical small molecule LC analysis. To permit access of large biomolecules to the stationary phase located in the intraparticle space, the typical pore size of 80–100 Å was increased to 1000 Å. Restricted pore access of mAbs can impact both retention and peak width (16,17); therefore, ensuring that the pore diameter is sufficiently large is a crucial aspect of stationary phase selection. The ability to install a wider variety of columns in the cartridge of the system used makes the adoption of wide-pore particles for this application much easier. To aid in protein recovery and reduce peak

TABLE 1: Comparison of deconvoluted masses (reported in Da) obtained using compact capillary LC–MS measurements in this study to comparable analytical-scale experiments reported in reference 15. (Note: Theoretical mass for intact trastuzumab [G0/G0F] is 147,911 Da)

	0.3 × 150mm (*This Study*)	[1.5/2.1] × 150mm (*Reference 15*)
Intact mAb (G0/G0F)	147,905	147,910
IdeS, Fc (G0F/G0F)	25,231	25,230
IdeS, Fab	97,628	97,628
Light Chain	23,441	23,446
Heavy Chain (G0F/G0F)	50,601	50,613

FIGURE 1: Mass chromatograms of (a) intact, (b) IdeS digested, and (c) reduced trastuzumab using the compact LC platform coupled to a Q Exactive HF mass spectrometer. Mobile phase A was water (with 0.1% DFA) and mobile phase B was 1:1 acetonitrile–*n*-propanol (with 0.1% DFA). The compact LC system was operated at 7 µL/min with gradients of 25–52.5%B over 5 min for (a), 20–50%B over 7 min for (b), and 10–67%B over 10 min for (c). Full mass spectrum and a zoomed-in spectrum of the +47, +48, and +49 charge states are shown in (d) and (e), respectively. Mass spectra are also shown for the (f) Fc, (g) Fab, (h) light-chain, and (i) heavy-chain fragments.



tailing, reversed-phase mAb methods are typically operated at temperatures in the range of 60–90 °C (18,19). A series of mass chromatograms showing intact, IdeS digested, and reduced trastuzumab samples are shown in Figure 1.

For these analyses, a 0.300 × 150 mm column packed with 2.7-µm 1000 Å diphenyl particles was installed into a column cartridge for instrument use. This wider pore material limited potential restricted access to pores for these larger biomolecules, especially for the ~150 kDa intact mAb. The heated cartridge oven was operated at 70 °C. The column was connected to an electrospray ionization probe for sample introduction into a high resolution mass spectrometer. The use of this LC–MS arrangement provided effective measurement of the molecular weights of the mAb and mAb fragments, aiding in the characterization of these compounds. A comparison of the deconvoluted masses for these peaks between the current study and previously reported analytical-scale experiments (15) is shown in Table 1. To improve peak shape, difluoroacetic acid (DFA) was used as the primary acidic modifier in the mobile phase instead of the more common LC–MS formic acid (FA) additive, as this substitution has been shown to reduce peak widths for mAb analysis (20,21). In addition, the composition of the organic component of the mobile phase was a 1:1 mixture of acetonitrile and *n*-propanol, as the addition of alcohol can further improve peak shape, especially when combined with elevated temperatures (18,22). An intact trastuzumab peak eluted with FA and acetonitrile is compared to elution using DFA and an acetonitrile–*n*-propanol blend in Figure 2. Such improvements that have already been observed with analytical-scale separations translate well to capillary LC.

Conclusions

Trends in the miniaturization of LC instruments provide for point-of-need analysis with a significant reduction in mobile phase consumption and waste generation through the use of capillary-scale techniques. Various parts of a typical LC–MS workflow for the analysis of biopharmaceutical products were demonstrated using this LC platform coupled directly to an MS system. This greener LC approach could eventually be used in biopharmaceutical manufacturing settings, especially if used for more routine monitoring of established processes with an absorbance detector that is much smaller than a typical MS system. The analysis of an intact mAb compared favourably between the compact instrument and a traditional benchtop LC system, showing the feasibility of eventually adopting these greener methods for routine biopharmaceutical characterization.

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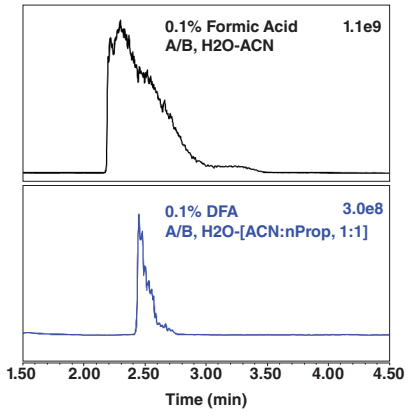
FIGURE 2: LC–MS analysis of intact trastuzamab using formic acid as modifier and acetonitrile as the organic mobile phase component (top black trace) compared to using difluoroacetic acid as modifier and 1:1 acetonitrile–*n*-propanol as the organic mobile phase component (bottom blue trace).



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