Mass Directed Fraction Collection for HPLC

TECHNICAL NOTE TN234

TECHNICAL FEATURES

- Multi-channel data collection
- Small footprint
- Positive/negative ion switching
- Clip-on ESI/APCI ion sources

TECHNICAL BENEFITS

- Mass monitoring allows for separation of non-UV active compounds.
- More affordable option for mass-based fraction collection.
- Addition of conditional fraction collection allows for more efficient workflows.

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In high performance liquid chromatography (HPLC), the selection of an appropriate mode of detection is vital to the success of any separation. To this end, there are numerous choices one might consider when configuring a new HPLC setup. Typically, the most popular choice among users is UV detection as it provides a balance between utility and cost. It allows for the monitoring of signals across a range of wavelengths and is ubiquitously employed in pharmacological research as proteins and oligonucleotides can be identified by monitoring different wavelengths. However, UV detection does have limitations. If the sample compound does not contain an active chromophore, it will be invisible to the detector. Additionally, UV detection is incapable of differentiating co-eluting compounds, and cannot be used to identify an unknown compound.

Figure 1
VERITY® 1920 Mass Spectrometer

Mass spectrometry (MS) is an alternative method of detection and identification that is much more robust than UV. It relies on a compound's specific mass rather than the presence of a chromophore, which gives more precise control over fraction collection than standard UV detection techniques. It can offer high precision and accuracy, but this high precision typically

comes with a much higher price tag. Consequently, while the capabilities of mass spectrometry continue to advance, this has done little to help put mass spectrometry as an inexpensive option for fraction collection until only recently.

The Gilson VERITY® 1920 Mass Spectrometer (VERITY 1920 MS) (Figure 1) has been added to our portfolio to give our customers access to MS detection capabilities at an affordable price for HPLC fraction collection. By sacrificing the high precision afforded by more expensive mass spectrometers in favor of ease of use, the VERITY 1920 MS offers a robust platform and can be outfitted with either electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) ion sources. Switching between ion sources is easy and can be done in a matter of seconds. Furthermore, the VERITY 1920 MS can scan in both positive and negative modes simultaneously, allowing for quick verification of unknown compounds. The VERITY 1920 MS also has a direct injection port for compounds that do not require chromatography beforehand. Documented below is an example of how the VERITY 1920 MS can add mass detection either as an alternative or as a supplement to UV detection for HPLC fraction collection.

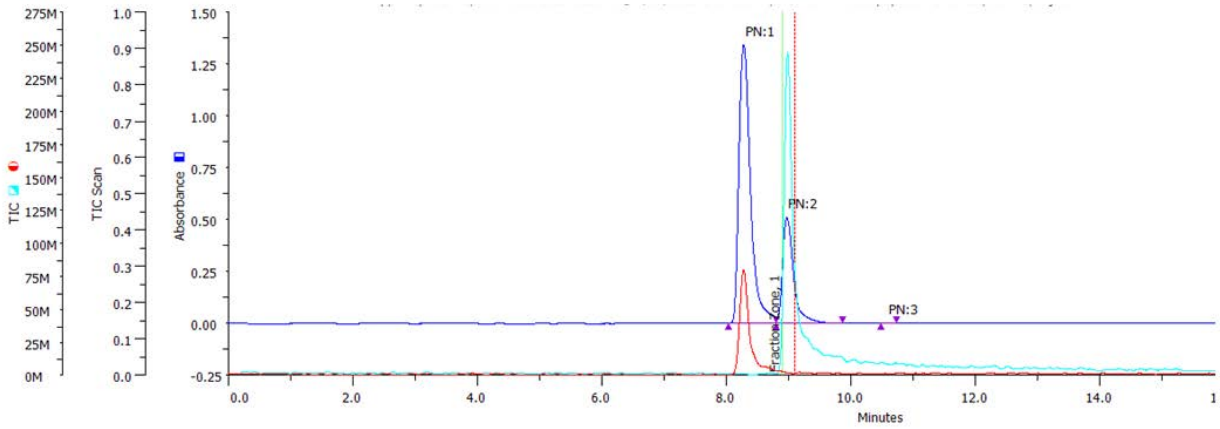

Figure 2

Chromatogram of caffeine/acetaminophen separation. UV trace (blue) plotted with selected mass ion monitoring traces of acetaminophen (red) and caffeine (cyan). By using mass directed fraction collection, only the caffeine peak was collected while the rest was directed to waste.

Using a previously described method, Figure 2 showcases the added flexibility mass directed fraction collection can offer. At equal concentrations, acetaminophen produces a stronger UV signal while caffeine produces a stronger mass signal. Using only UV-triggered fraction collection you would be able to collect both compounds or just acetaminophen if a high enough threshold is set; however, with this mixture, it would be impossible to only collect caffeine due to its lower signal compared to acetaminophen. With the addition of the VERITY 1920 MS and the ability to use mass detection to supplement or as an alternative to UV detection, fraction collection settings can be further fine-tuned to exclude peaks of non-interest from collection. By collecting based on mass, only the caffeine peak is collected even though its UV signal is less than acetaminophen's.

While the VERITY 1920 MS has a scan range of up to 2000 m/z, Gilson software can detect multiple charged species which can increase the effective molecular weight detection limit. In separation runs using a mixture of two oligopeptides, fraction collection of a single peak using mass directed collection was still accomplished without the presence of a singular charged

molecular ion. Figure 3 shows that even though the positive molecular ion m/z=1300 for a mass 1299 peptide is not detected, the presence of the doubly charged m/z=650 can still trigger fraction collection.

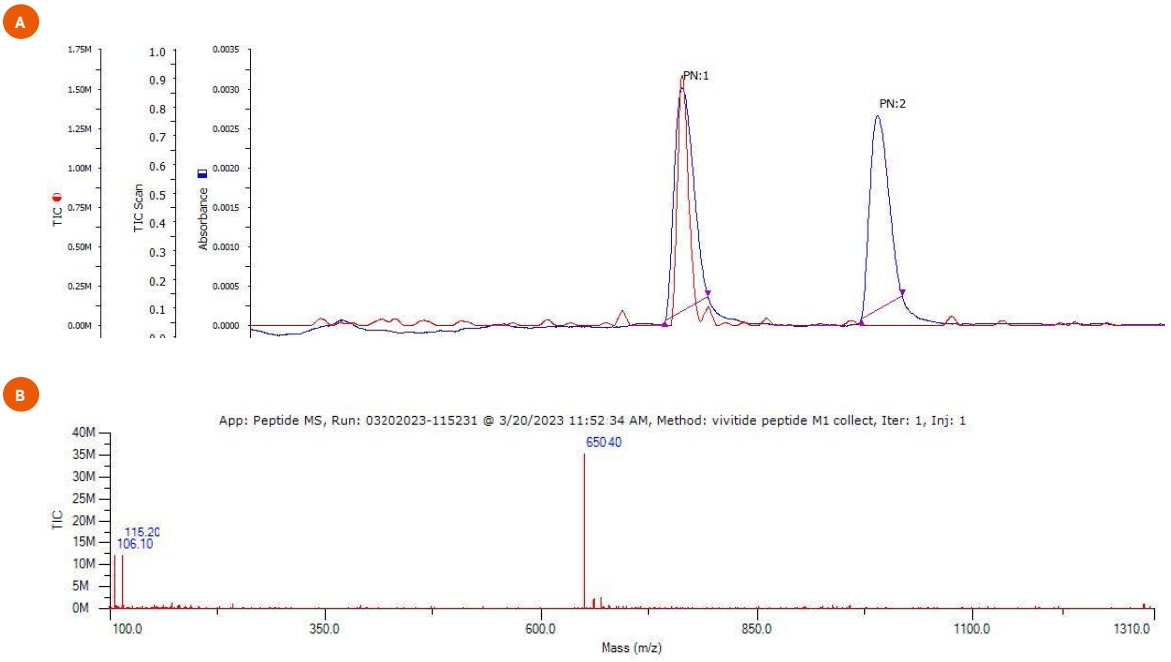

Figure 3

(A) UV chromatogram (blue) overlayed with selected ion monitoring chromatogram (red). Targeted mass for red trace was 1299. (B) Mass spectrogram of first peak containing a nm=1299 peptide. While the singly charged molecular ion is not present, the doubly charged m/z=650 is.

The VERITY 1920 MS has been developed to be an affordable option for the identification of compounds through mass. While it can be used as a stand-alone instrument for mass verification without purification, it offers significant benefits when coupled with an HPLC separation system:

- Increased efficiency and flexibility when developing separation and purification methods.
- Easy-to-change ion sources allow for faster method development.
- Charge detection allows for the collection of large, highly charged species and oligomeric compounds.
- Ion switching allows for increased detection capabilities and more advanced fraction collections achievable in a single run.

For more information about the VERITY 1920 MS please contact us or your local Gilson account manager.