

Using LC-MALDI-MS to Study the Variability and Stability of Plasma Proteins

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Overview

Purpose

Compare plasma peptidome from EDTA vs. BD™ P100*.

Methods

- Collect blood in EDTA and P100 Tubes.
- Centrifuge for 10 min at 2,500xg allowing the mechanical separator to isolate the plasma from blood cells.
- Plasma was aliquoted at various time intervals.
- The peptidome was recovered using a 3kDa MWCO filter.
- Peptides were cleaned with two Ziptips each and pooled for LC-MALDI-MS.
- Virtual 2-D plots were created enabling the direct comparison of EDTA and P100 plasma peptidomes.

Result

- Successful comparison of plasma peptidome from EDTA vs. P100 Tubes.
- Presence of protease inhibitors in the BD™ P100 tubes stabilizes both native proteins and peptides.

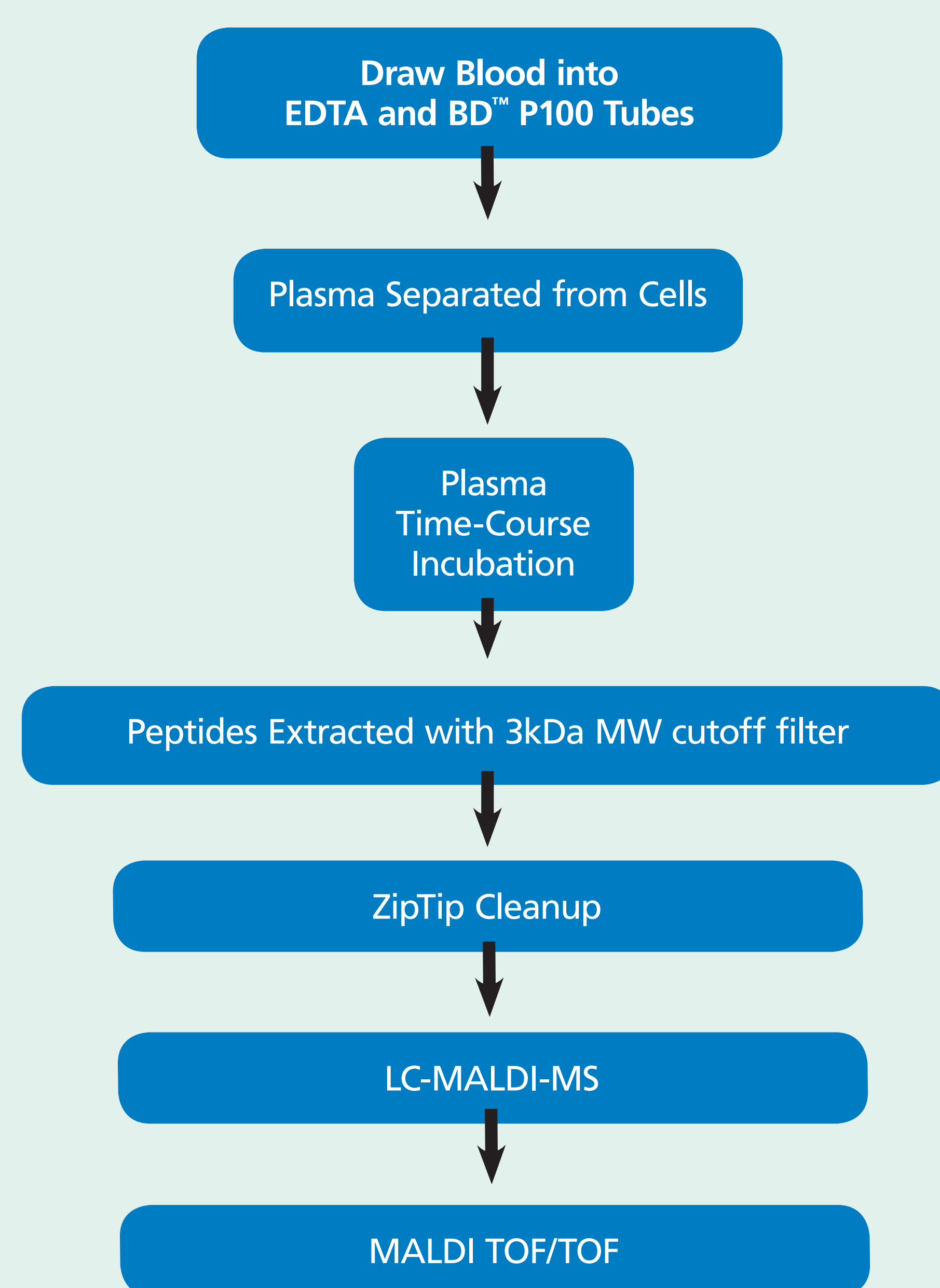
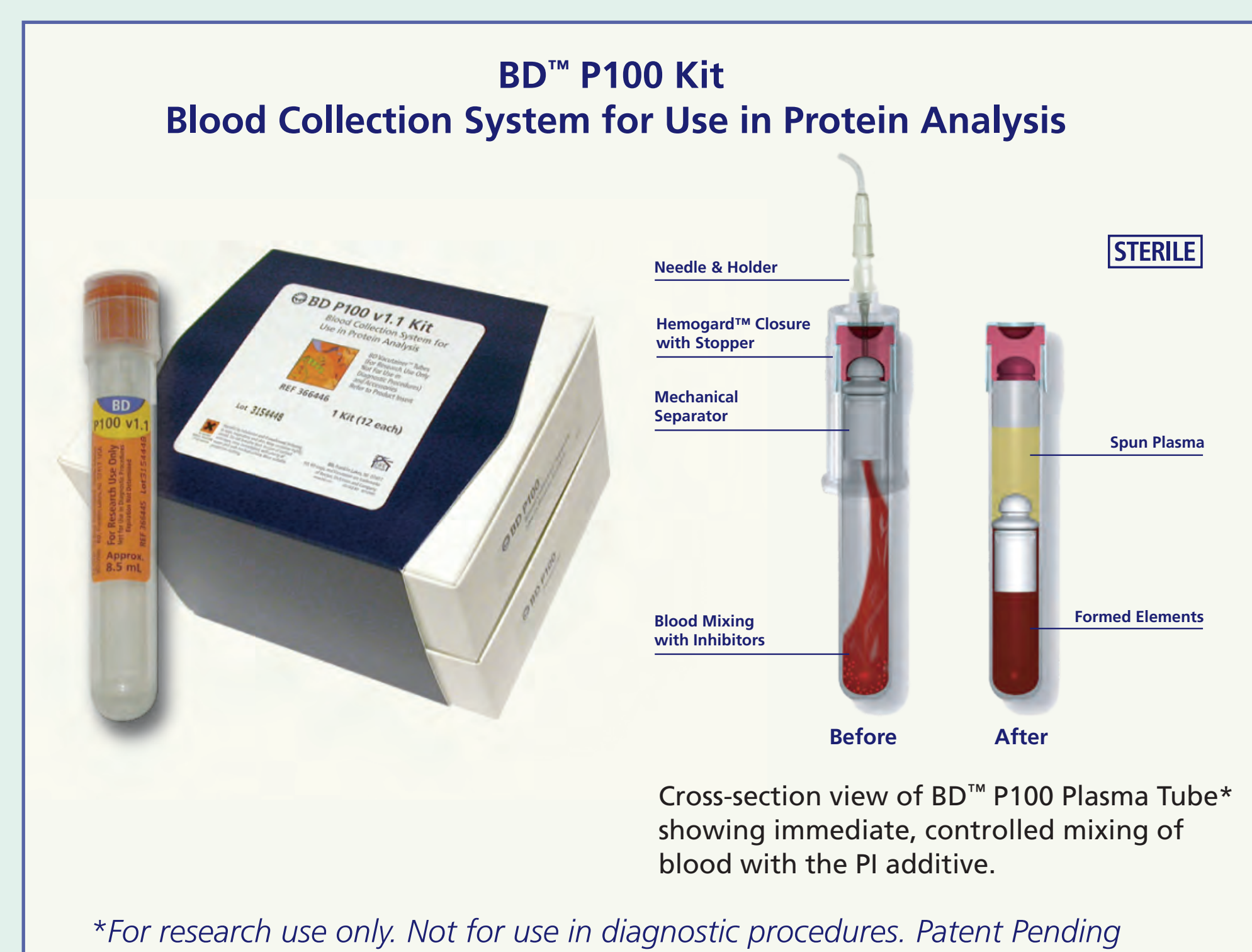
Introduction

The search for proteomic biomarkers from human plasma holds both incredible clinical potential as well as significant challenges.¹ The dynamic range of concentration, known to exceed ten orders of magnitude, is the primary limitations in plasma proteomic analyses, typically limited to 10⁴-10⁶ with current instrumentation. Beyond the well-known dynamic range issues, plasma proteome analysis is further complicated by preanalytical variability, in particular, during blood collection and early sample handling. These sample processing issues need to be evaluated.

Our previous experiments demonstrated that plasma, collected in evacuated blood tubes including protease inhibitors present at the moment of phlebotomy, yields more time-stable and intact samples.² Standard serum and anticoagulated plasma samples, in parallel studies, are measurably less stable. After centrifuging, separating plasma from blood cells, the samples were first incubated for different lengths of time, then passed through 3kDa molecular weight (MW) cutoff filters. The resulting peptides were analyzed by Matrix-Assisted Laser Desorption/Ionization-Mass Spectrometry (MALDI-MS). MS results indicate that "new" peptides were being generated *ex vivo* more rapidly in standard EDTA tubes, as compared to measurably increased stability using protease inhibitors (BD™ P100* Tubes).

In the previous studies, the entire peptide content from each sample was analyzed by direct MALDI-MS. In the current study, we increased the dynamic range of detectable peptides by using reversed-phase chromatography, in a LC-MALDI-MS format. Methodical probing of plasma peptides enables deeper understanding of preanalytical variables associated with sample collection and handling. It further elucidates the beneficial aspect of *in vitro* protease inhibitors and their role in establishing plasma proteome sample acquisition and handling standards.

Experimental



To increase reproducibility of LC-MALDI-MS:

- Five MWCO filters were prepared in parallel and pooled.
- Prespotted AnchorChips from Bruker were used.
- Each time interval was run in duplicate.

Virtual 2D plots and UV chromatographs are displayed in Figure 1. Comparing the peptidome from P100 and EDTA tubes directly, the virtual 2D plots appear complex and complicated (Figure 1). However, a couple of key features stand out.

First, UV chromatographs of the replicate runs are very consistent to each other. Second, the virtual 2D plots created by the LC-MALDI-MS were run in duplicate to assess inherent process variability of LC-MALDI. The 2D plots are very reproducible for MALDI, especially compared between time points.

Results

Figure 1. Monitoring peptide variations in EDTA and BD™P100 over time with LC-UV and LC-MALDI-MS

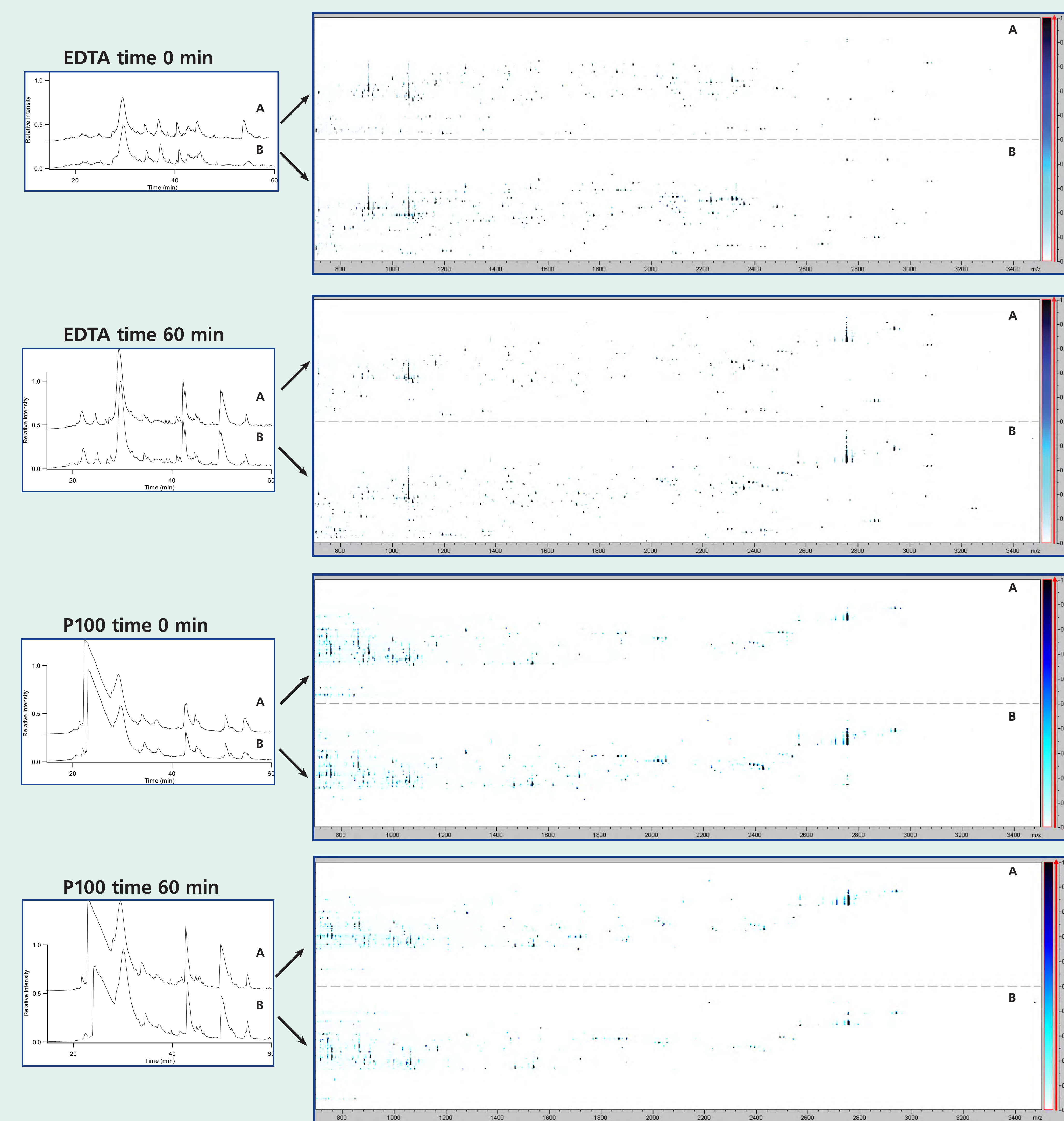


Figure 1 displays UV chromatographs and virtual 2D plots of the LC-MALDI-MS data.

- Chromatograph for EDTA time 0min is very different from the same sample at 60 min.
- Conversely, P100 at time 0 min is very similar to the same sample at time 60 min.
- There are more peptide variations between the EDTA tubes over one hour compared to P100, particularly in the 750 - 900 *m/z* and 2000 - 2500 *m/z* regions.
- P100 plasma peptidome is more stable over time than a standard EDTA plasma sample.
- It should also be noted that P100 has less peptides at time 0 and 60 compared to EDTA at its respective time intervals.
- The increase in EDTA detected peptides is a result of proteolytic activity in the EDTA tube.
- Therefore the protease inhibitors in P100 protect the sample both at the moment of collection and also over time.

Post Source Decay (PSD) was performed on highly abundant peptides of both peptidomes obtained from EDTA and P100 using lift mode on the ultraflex (Bruker Daltonics, Germany) for the 60 minute time interval LC-MALDI fractions. Several high abundant proteins, including albumin, apolipoprotein, fibrinogen, and antitrypsin were identified in both the EDTA and P100 Tubes. However, more peptides from these proteins were identified in the EDTA tube. The C-terminus amino acids of the identified peptides varied, suggesting these are generated by different proteolytic pathways. The combination of results demonstrate a greater protein stabilization beginning at the time of blood collection and over time in the P100 Tube vs. EDTA. Thus, P100 displays less peptide variation over time.

Protein	MASCOT Score	Number of Peptides
Albumin	410	10
Hypothetical Protein Hflu	181	4
Chain D, Apolipoprotein	142	6
Fibrinogen	111	3
Chain B Alpha-1 antitrypsin	96	2

In previous work, we have used direct MALDI to detect the relative amount of bradykinin present in plasma in both EDTA and P100 tubes. These experiments indicated that P100 stabilizes bradykinin (1060 *m/z*) during and after blood collection. However, our data in Figure 1 show that bradykinin is only 10X lower in P100 vs EDTA, atypical of results with much lower levels in P100 from dozens of other samples we have tested. We suspect elevated bradykinin in Figure 1 to represent a donor specific event. Blood was collected from a healthy donor in both P100 and EDTA tubes, and processed as previously described. Figure 2 shows LC-MALDI data much more consistent with our typical direct MALDI observations.

Figure 2. Peptide Bradykinin (1060.5 *m/z*) in P100 vs. EDTA

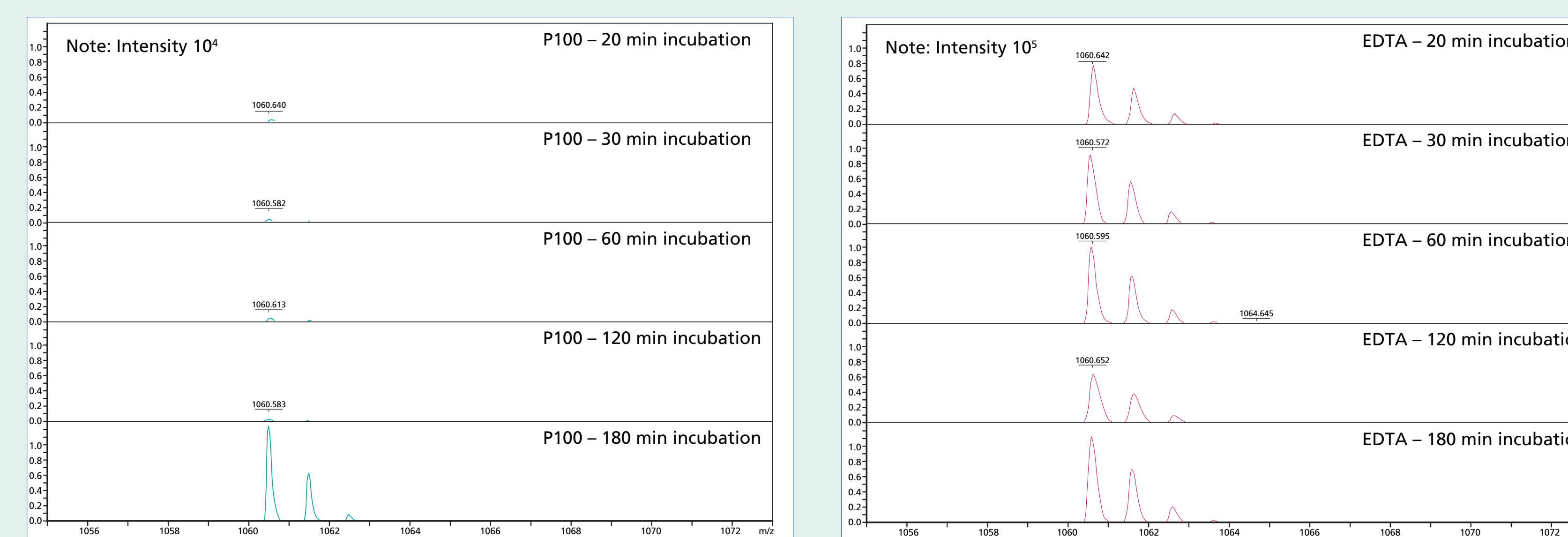


Figure 2 displays bradykinin from the most abundant LC-MALDI fraction at different time intervals.

- Kininogen appears stable in the P100 tube for at least 120 min, resulting in a low amount of Bradykinin detected.
- It also suggests this peptide is being produced in the EDTA tube during and after blood collection.
- Typically, Bradykinin arises as a post-draw artifact³.

Conclusions

- Reproducible LC-MALDI-MS virtual 2D plots were created to compare P100 and EDTA tubes.
- Protease inhibitors present in the BD™ P100 Tube increase both protein and endogenous peptide stability starting at the time of blood collection.
- Observation of more anomalous peptides in EDTA plasma at both 0 and 60 mins compared to P100 samples demonstrates greater protein stabilization in P100 as a result of the protease inhibitors.
- More peptides detected in EDTA as a result of *ex vivo* protease activity.
- P100 may stabilize Kininogen protein containing peptide Bradykinin (1060 *m/z*) at the time of blood collection.

References:

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