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Nota de aplicación

Multi-Enzyme Digestion for Biotherapeutic Peptide Mapping: Examining BiopharmaLynx 1.3 Functionality

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application note demonstrates advanced multi-protease peptide mapping workflows, now enabled with the automated data analysis capabilities of the BiopharmaLynx Application Manager, version 1.3, for MassLynx Software.

Benefits

Enabling advanced multi-protease peptide mapping workflows, with the automated data analysis capabilities of the BiopharmaLynx Application Manager, version 1.3, for MassLynx Software.

Introduction

The recent availability of high-quality proteolytic digestion enzymes, in addition to trypsin, has facilitated a

new generation of biotherapeutic peptide map analyses that can be performed with much greater flexibility.

In particular, multiple protease digestion workflows have enabled greater selectivity for peptide chromatographic retention, MS response, and peptide fragmentation pathways, while simplifying the task of achieving comprehensive sequence and fragmentation coverage for a given biotherapeutic. This technical brief will describe the employment of such workflows, and how they can yield greater biotherapeutic knowledge, often with less analytical development effort.

Results and Discussion

The most recent release of BiopharmaLynx, version 1.3, features additional flexibility for automating data analysis from higher complexity multi-enzyme peptide map experiments. BiopharmaLynx supports maps generated with the common proteolytic digest reagents (Table 1), and also provides scientists the flexibility to define additional custom digest reagents. BiopharmaLynx 1.3 now extends bioinformatic support for peptide mapping to analyses where multiple digestion enzymes are utilized.

Enzyme	Code	Selectivity	Digest	Exclusions
Arg-C	R	R	C-terminal	R(P)
Asp-N (Asp)	D	D	N-terminal	None
Asp-N (Asp and Glu)	D	D, E	N-terminal	None
CNBr	M	M	C-terminal	None
GluC (V8, NH4OAc pH 4)	V	E	C-terminal	E(P)
GluC (V8, AmBiC pH 7.8)	V	E	C-terminal	E(P)
GluC (V8, PO4 pH 7.8)	V	D, E	C-terminal	D(P), E(P)
Chymotrypsin	C	Y, F, W	C-terminal	Y(P), F(P), W(P)
Lys-C	K	K	C-terminal	K(P)
Lys-C (w/o Pro Exclusion)	K	K	C-terminal	None
None	N	None	None	None
Non-specific	F	All Bonds	C-terminal	None
Slymotrypsin	S	K, R, Y, F, W	C-terminal	K(P), R(P), Y(P), F(P), W(P)
Trypsin	T	K or R	C-terminal	K(P), R(P)
Trypsin (w/o Pro Exclusion)	T	K or R	C-terminal	None
Custom digest reagents	User	User	User	User

Two multi-digest workflows are supported within the peptide mapping method editor (Figure 1): the combined workflow (or “one pot” digest), where multi-protease digestion is carried out in the same sample vial; and the separate workflow (or “or multi-pot” digest), where individual enzyme digests are prepared, quenched, and then mixed prior to LC-MS analysis.

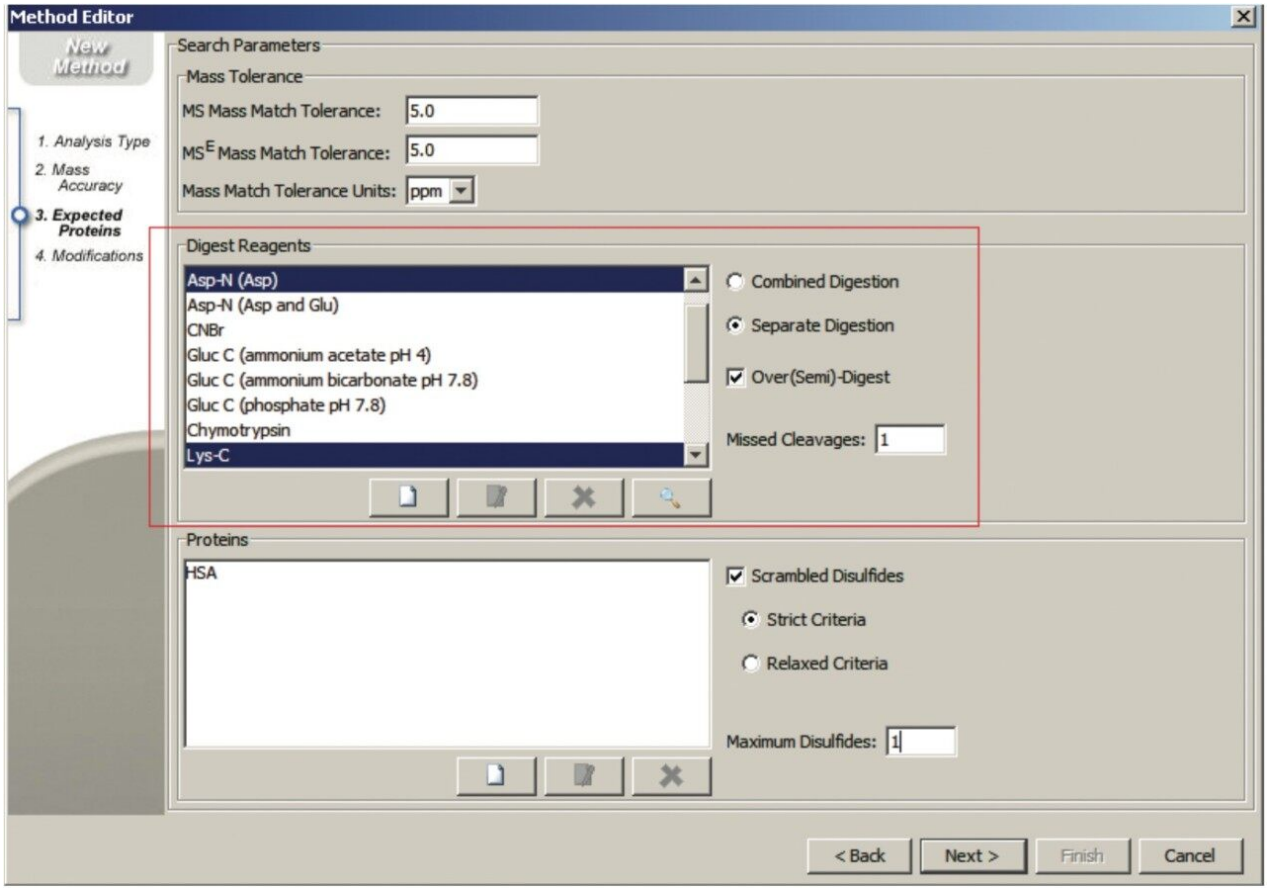

Figure 1. Digest reagent selection within the BiopharmaLynx 1.3 method editor. Users can now select multiple digestion enzyme in addition to overdigestion and underdigestion products for those proteases.

Figure 2 illustrates the digest specificity on a theoretical protein from individual digestion with GluC and LysC. Individually, they each produce three digested peptides, including one very large peptide. T he products of the separate multi-enzymatic workflow represent the concatenated list from the individual digests, while the products of the combined multi-enzymatic workflow constitute a unique set of digested peptides, where the large peptides have been further digested by the additional protease.

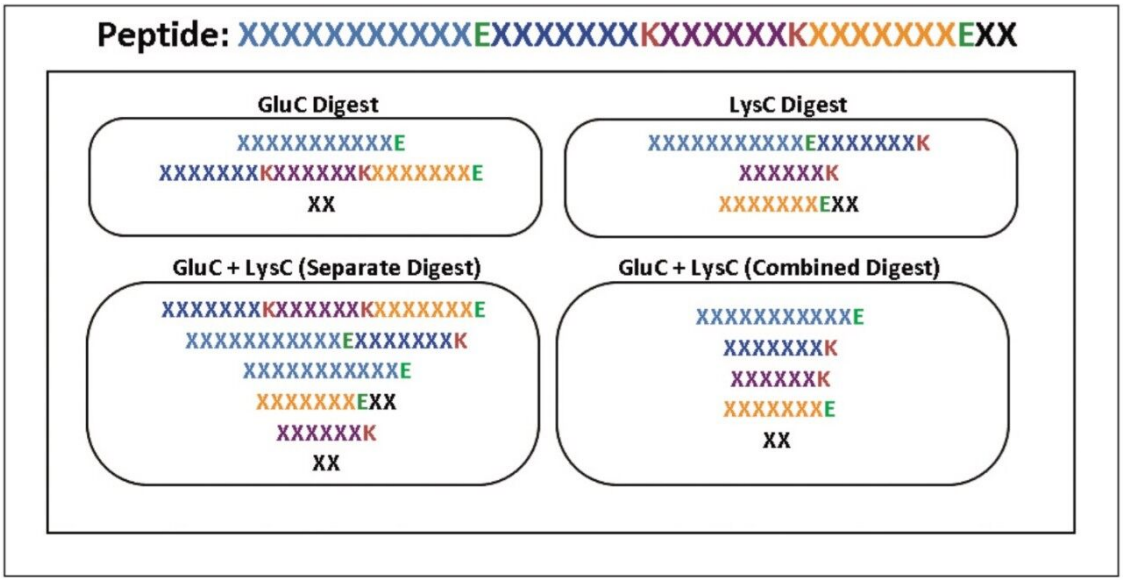

Figure 2. Digestion specificities for single, multi-combined, and multi-separate proteolytic digest workflows.

The following sections will detail the practical utility of these new workflows for biotherapeutic peptide mapping analysis.

Combined multi-digest workflow

The combined workflow generates a set of peptides resulting from the combined specificities of all proteases used in the digestion process. Identified peptides are labeled using multi-letter peptide digest labels derived from the single enzyme digest designators (e.g., DT represents the combined product of AspN(D) and Trypsin (T)).

There are three common reasons to utilize the combined digest multi-enzyme workflow:

- To change the peptide digest population, altering chromatographic properties of the mixture. This will often introduce significant changes in retention time for multiple peptides, and can address peptide coelution, or improve chromatographic quality for one or more components.
- To reduce large peptides obtained from a single enzyme digest to more manageable-sized peptides for fragmentation studies. It can be difficult to generate fragmentation at all peptide bonds when sequencing larger peptides (> 25 AA). Reducing peptide size can often enhance fragment coverage for peptides of interest, and can be particular useful for producing smaller peptides where multiple modifications can be monitored independently.

- Changes to the terminal amino acid residues can significantly alter peptide fragmentation behavior and allow more confirmatory ions from sequence regions not favored for fragmentation of the larger peptide.

Separate multi-digest workflow

The separate workflow assumes that multiple independent digests were produced and that the enzymes were inactivated before the digests were mixed for analysis. BiopharmaLynx searches peptide mapping results for peptides predicted using the digest specificities of each enzyme. Identified peptides are labeled using the single-letter peptide digest nomenclature common to single enzyme digests (e.g., T for Trypsin; K for LysC; D for AspN)

There are three common reasons to utilize the separate digest multi-enzyme workflow:

- To obtain high sequence coverage with minimal method optimization for a given protein. This could prove useful for discovery scientists faced with evaluating large numbers of protein candidates, or during clone selection for a particular candidate.
- To obtain redundant protein coverage from a single peptide map. Modifications can be independently confirmed and quantitated using overlapping peptide sequences from each digest.
- To maximize sequence confirmation from peptide fragmentation using fragmentation selectivity differences between digested peptides covering a common sequence region.

Applying the separate digest workflow to compare digestion enzymes

The new separate digest workflow functionality can also be employed to compare maps prepared with two different enzymes. As this workflow generates concatenated lists of both enzyme digest products, searches against both data sets would return accurate identifications.

Human Serum Albumin (HSA) was individually digested with GluC and LysC, and high quality UPLC-MS^E maps were generated for both digests. The annotated Total Ion Chromatogram (TIC) traces for both runs (Figure 3) show peptide identifications for fully digested products in both digests.

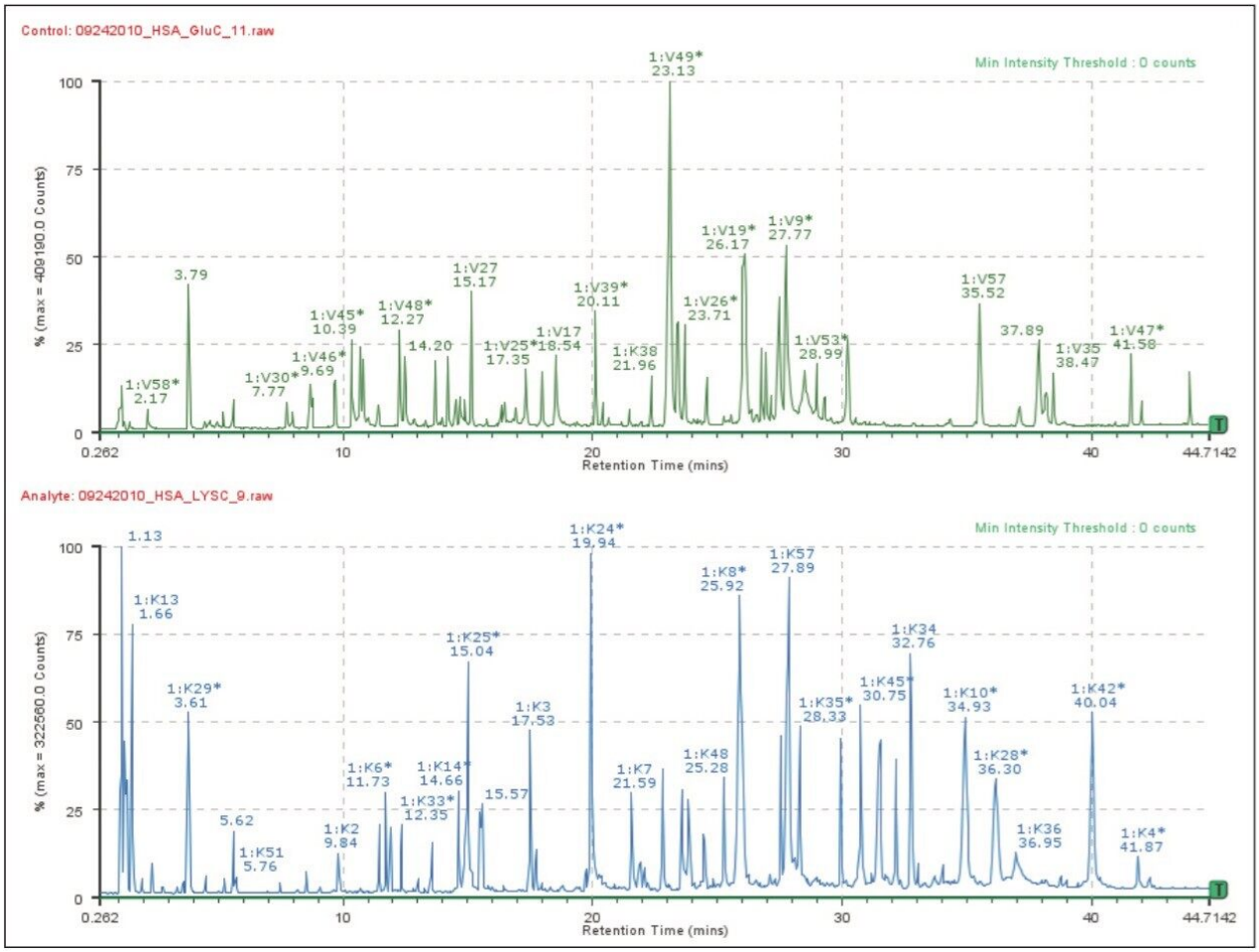

Figure 3. Peptide mapping results (TIC) for Human Serum Albumin (HSA) digested with GluC (top, V labels) and LysC (bottom, K labels).

Without any optimization, it was shown (Figure 4) that 85% coverage was obtained with GluC, 92% coverage with LysC, and 100% sequence coverage from the combined results of both analyses. The absence of "common" coverage reflects the lack of identical peptide fragments generated from both enzyme digests, and the excellent specificity of accurate mass for biotherapeutic peptide mapping studies. From such studies, useful combinations of enzymes can be readily identified to assist in answering global or targeted questions about a biotherapeutic protein.

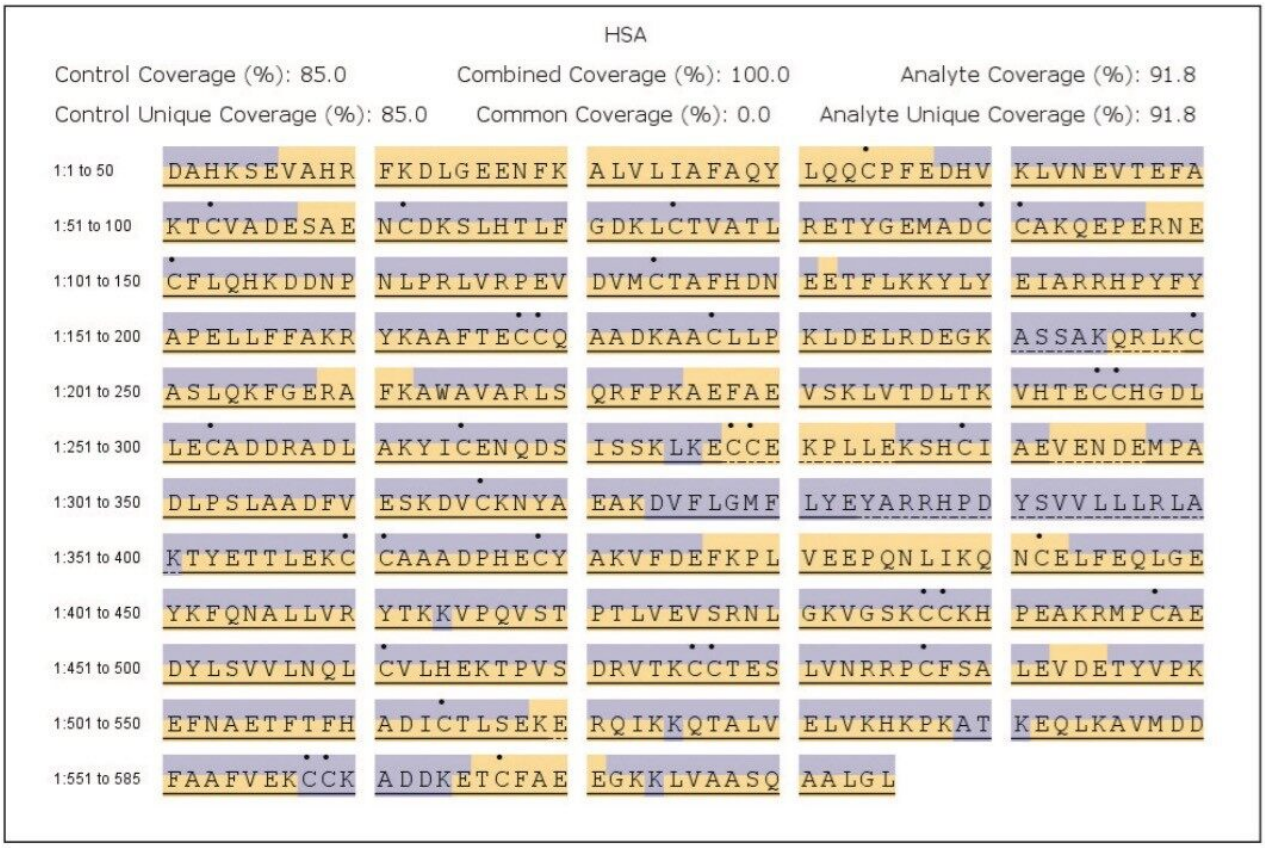

Figure 4. Coverage plot showing GluC (purple) and LysC (orange) coverage for HSA digested with the specified enzyme in a single enzyme digest.

Conclusion

- BiopharmaLynx 1.3 now supports multi-enzyme LC-MS peptide mapping analysis workflows.
- Such workflows can be employed to alter chromatographic and MS selectivity, to address issues of map coverage, and to enable more comprehensive studies of biotherapeutic variation.
- The separate digest functionality in BiopharmaLynx 1.3 can also be used to rapidly evaluate map coverage and quality for various protein digestion enzymes.

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