Automated Optimization of LC/MS Peptide Mapping Methods

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OVERVIEW

- Peptide mapping has become a defacto "gold standard" to assess the identity, stability, and batch-to batch consistency of protein therapeutics.
- Optimization of LC eluent composition plays an important role at the early stages of method development, as each chromatographic sorbent will have differing selectivity and performance based on the gradient and acidic modifier selected.
- In LC/MS methods, the positive chromatographic performance of an acidic modifier must be balanced with the potential for suppressing electrospray ionization.
- In this poster, we will show how an automated methodology (AutoBlend™) permits rapid method development for peptide mapping studies, and how the balance between chromatographic performance and MS response can be optimized with minimal user intervention.

METHODS

Figure 1: LC/ESI-TOF MS Configurations.

Instrumentation: An Alliance® 2796 Bioseparations System was configured with a 2487 dual wavelength UV detector (containing an inert biocompatible analytical flow cell), and a Micromass® LCT™ ESI-Tof MS (Waters). A post-column split (1:6 MS to UV) permitted simultaneous MS and UV analysis. A MassLynx™ 4.0 workstation was used for instrument control, data acquisition, and data processing.

Chromatography: All experiments were carried out with an BioSuite™ PA-A (2.1 x 150 mm) column (Waters) at a flow rate of 0.5 ml/min. Gradients of 2 to 48% acetonitrile were run over 86 min., with the concentration of the acidic modifier decreasing linearly over this period by 20% of the starting concentration.

Sample: MassPREP™ Enolase digestion standard (Waters) was dissolved in either 0.1% Formic acid or 0.1% TFA to a final concentration of 5 pmol/µl for these experiments. 65 µl (320 pmol) was loaded for each analysis.

Autoblend Methodology

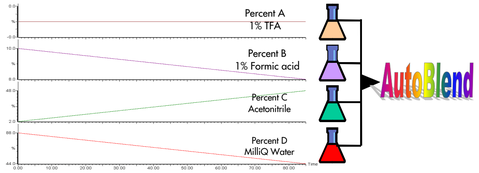

Figure 2: Autoblend Methodology. The AutoBlend approach involves online blending of four or more chromatographic inlet channels to produce a resulting eluent mixture. In this work, four inlet solutions (1% TFA, 1% Formic acid, Acetonitrile, and Water) are mixed to permit generation of RP gradients with adjustable levels of two acidic modifiers. In this example, a 2-48% acetonitrile gradient is developed in a background of 0. to 0.08% Formic acid during the course of the run.

RESULTS

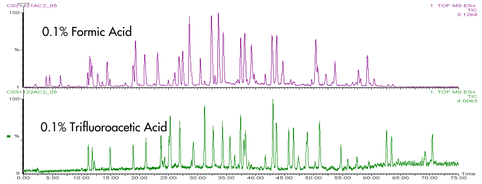

Figure 3: The choice of acidic modifier will have significant effects on the pattern of a peptide map. The Total Ion Chromatograms of an enolase digest were generated in 0.1% Formic acid (Top) and 0.1% Trifluoroacetic acid (Bottom) are presented. The acidic modifier has a noticeable effect on the overall peak pattern, retention, width and shape.

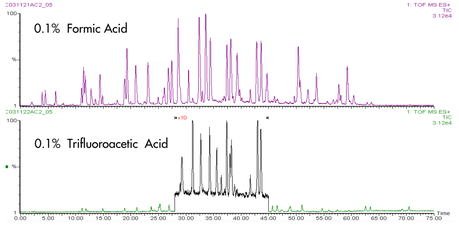

Figure 4: Acidic modifiers can have a significant effect on ESI-MS response. Equal loadings of the digest were analyzed under 0.1% FA (Top) and 0.1% TFA (Bottom) conditions. TIC traces for the two runs (linked y-axes) clearly show TFA induced suppression (~90%) of the ESI-MS signal.

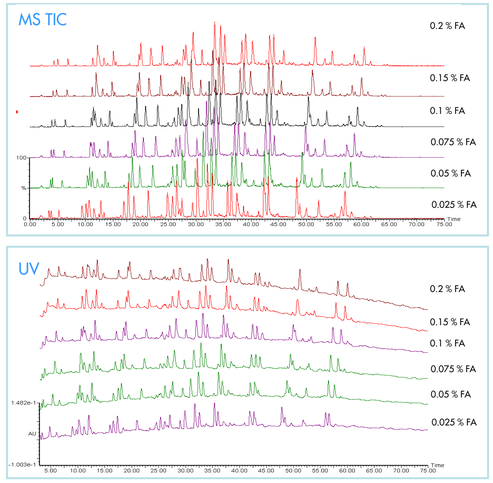

Figure 5: MS TIC (Top) and UV$_{215nm}$ (Bottom) peptide maps generated using increasing amounts of formic acid (linked y-axes). With increasing acid concentration, overall retention is slightly increased, and no significant UV or MS signal suppression is observed .

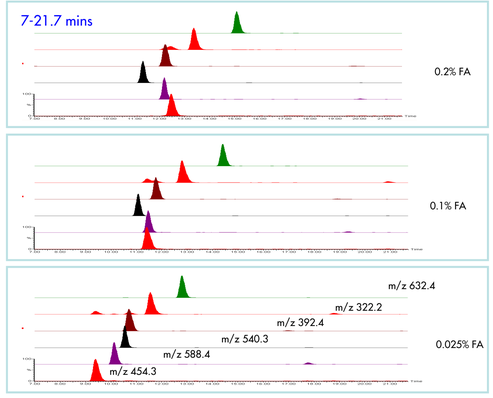

Figure 6: Extracted Ion Chromatograms of selected enolase peptides show significant differences in peptide elution patterns depending on the concentration of Formic acid. Six different peptide masses were selected to show the change in elution pattern with increasing formic acid concentrations. The increase of FA content caused a higher hydrophobicity of the peptides which result in longer retention times.

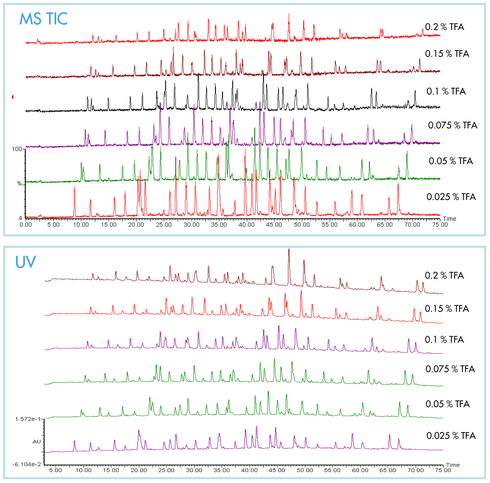

Figure 7: MS TIC (Top) and UV$_{215nm}$ (Bottom) peptide maps generated using increasing amounts of TFA (linked y-axes). With increasing acid concentration, overall retention is slightly increased, and decreasing MS signal is observed while the UV peak areas remain relatively unaffected.

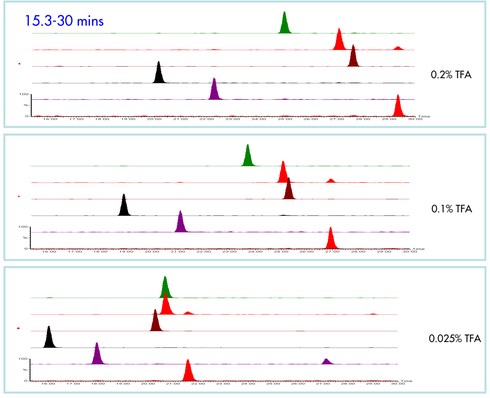

Figure 8: Extracted Ion Chromatograms of selected enolase peptides show significant differences in peptide elution patterns depending on the concentration of TFA. Six different peptide masses were selected to show the change in elution pattern with increasing TFA concentrations. Note that elution order of these peptides varies significantly with both the choice and concentration of the acidic modifier.

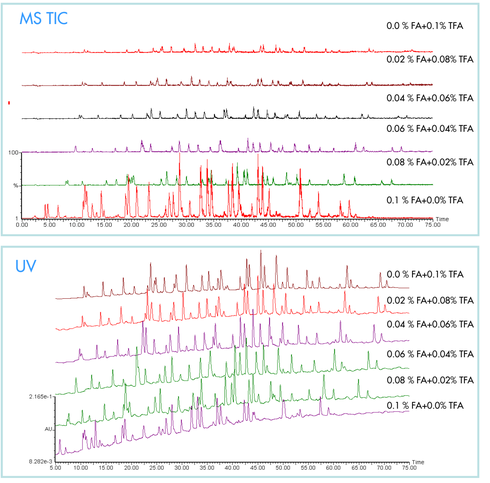

Figure 9: MS TIC (Top) and UV chromatograms (Bottom) of the enolase peptide map using mixed acid modifiers (linked y-axes). The total percent of acid modifier was kept at 0.1%, but the ratio of the two modifiers was adjusted as indicated. ESI-MS signal suppression occurs very rapidly upon addition of TFA, and continues to increase as TFA concentration is increased. The pattern of the map using these hybrid modifiers is different than maps using either modifier alone.

CONCLUSIONS

- The AutoBlend™ methodology permits rapid automated development of LC/UV and LC/MS peptide mapping methods.
- The choice of acidic modifier as well as the selected concentration of the modifier will have profound effects on a peptide map pattern.
- For the Biosuite™ PA-A chemistry tested in this work, both FA and TFA are effective modifiers when used at a wide range of concentrations.
- FA could be used at a variety of concentrations without affecting UV or MS sensitivity, while TFA significantly suppress only ESI signal.
- The electrospray suppression effects of TFA are dominant when mixing both TFA and FA.
- The ability to blend multiple acidic modifiers during segments of an LC analysis may be useful to alter separation selectivity to further resolve complex regions of a peptide map.