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Automated Optimization of Intact Protein LC/MS Analyses

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Overview

- Analysis of intact proteins by MS is a powerful approach for protein identification and assignment of protein modifications.
- Analyzing protein mixtures typically requires chromatographic processing to separate, concentrate, and desalt components prior to MS detection.
- Optimization of eluent composition plays an important role in LC/MS methods development, as sorbents possess differing selectivity and performance based on gradient shape and choice of acidic modifier.
- The positive chromatographic performance of an acidic modifier must be balanced with the potential for suppressing electrospray ionization.
- This poster describes a methodology where separation and ionization issues for protein LC/MS analysis are addressed in an efficient and automated fashion.

Methods

System: Waters® Alliance™ Bioseparations System composed of a 2796 Bioseparations Module, Column Heater Module, 2996 Photodiode Array Detector, ZQ™ Mass Detector, and MassLynx™ Data System.

Chromatography: BioSuite™ pPhenyl Column (2.0 x 100mm, 1000A, 10µ polymeric particle) and Symmetry300™ C_{18} Column (2.1 x 75mm, 300A, 5µ silica particle) operated at 150 µl/min. Separations began with a 5 min hold at 5% acetonitrile, followed by a 30 min linear gradient to 90% acetonitrile, maintaining a constant concentration of acidic modifier.

Sample: A mixture of seven proteins [bovine insulin (4 µg, 6 kD), horse cytochrome c (3 µg, 12 kD), bovine ribonuclease A (16 µg, 14 kD), horse myoglobin (2 µg, 17 kD), yeast enolase (3 µg, 47 kD), BSA (6 µg, 66 kD, apo-Transferrin (6 µg, 80 kD)) were prepared in 5% acetonitrile, and injected (10 µl) for each run.

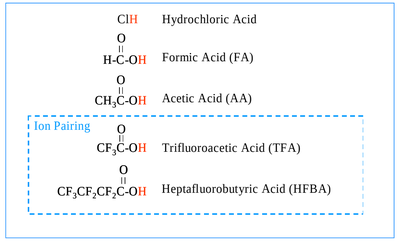

Figure 1: Acidic Modifiers for Reversed Phase LC/MS Separations. Acidic modifiers have several roles in an LC separation including, the ability to produce uniform positive charge on analytes, provide buffering capacity to maintain reproducible separations, and modulate analyte-sorbent interactions. While all acids bind electrostatically to the analyte, ion-pairing agents also interact with both analyte and sorbent through hydrophobic associations.

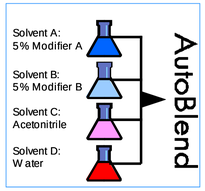

Figure 2: AutoBlend™ Methodology. The real time quaternary blending of water, acetonitrile, and various acidic modifiers can produce isocratic and gradient reversed phase separations with precise control of organic and modifier concentration. Thus a series of runs can be constructed to automate optimizing the composition, length, and shape of a reversed phase separation.

Results

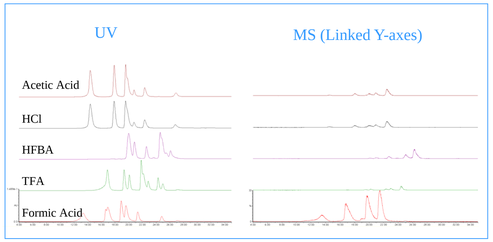

Figure 3: Comparison of Acidic Modifiers for Protein LC/MS Separations-Suppression Issues. A six protein mixture was analyzed by LC (polymeric RP column)/MS in the presence of 0.1% of the indicated acidic modifiers. The A_{280} UV chromatogram **(LEFT)** shows the resulting separation profiles for each chromatographic system. The MS TIC patterns **(RIGHT)** show the dramatic effects of ion suppression from all modifiers other than formic acid.

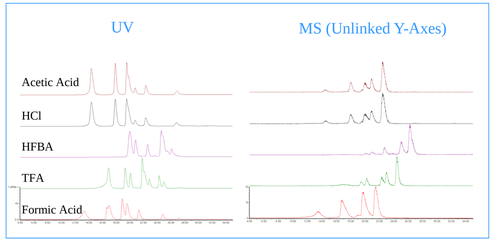

Figure 4: Comparison of Acidic Modifiers for Protein LC/MS Separations-Chromatographic Issues. If the y-axis for each run is independently scaled, we can see from both the UV and MS traces that the choice of acidic modifier will have noticeable effects on peak retention time, column selectivity, and chromatographic resolution.

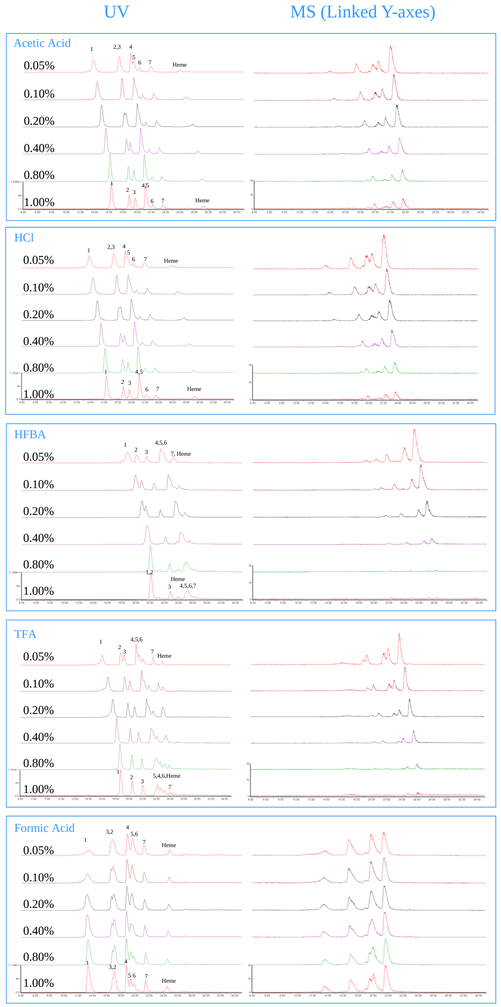

Figure 5: Effects of Acidic Modifier Concentration on LC/MS Separations. Varying acidic modifier concentration affects both the quality of the separation, and the extent of signal suppression. Proteins: (1) Rnase A, (2) Insulin, (3) CytC, (4) apo-transferrin, (5) BSA, (6) Myoglobin, (7) Enolase.

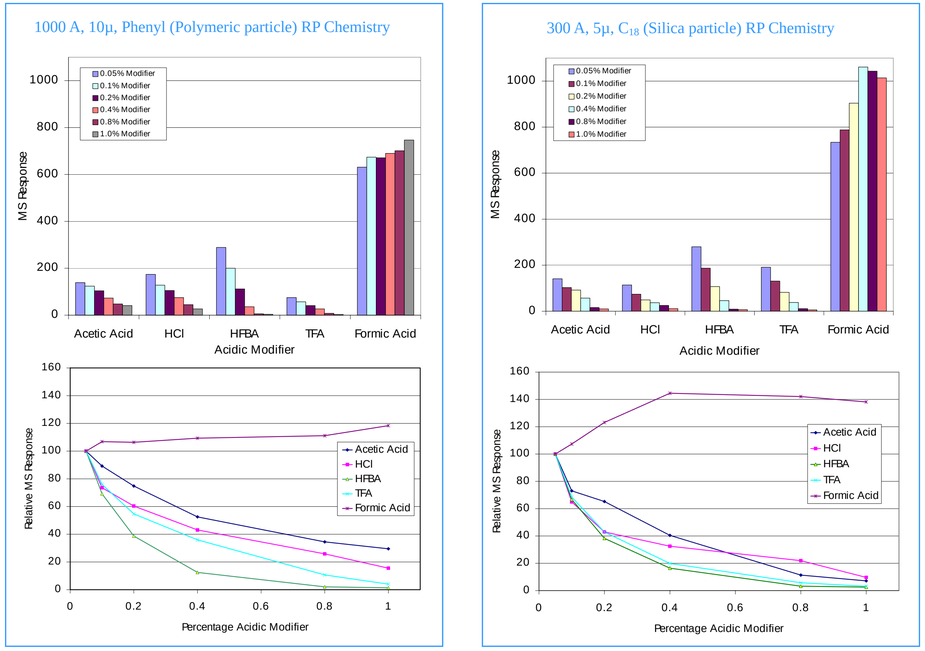

Figure 6: Comparison of various acidic modifiers for LC/MS performance. The peak areas for the latest eluting component (Enolase) were determined for LC/MS analyses using a polymeric (LEFT), and silica based (RIGHT) reversed phase chemistry under a variety of acidic modifier concentrations. Similar performance is observed with both chemistries under the various conditions, except that MS response from the silica based column is more significantly improved with intermediate formic acid concentrations. Other acids show only increasing suppression with increasing concentration.

Conclusions

- An AutoBlend™ methodology permitted testing multiple LC/MS separation methods in an automated fashion.
- The type and concentration of an acidic modifier produce significant MS sensitivity and LC separation quality effects.
- The MS suppression effects are generally comparable for both polymeric and silica based RP separation chemistries.
- Formic acid demonstrates the least suppression of the acids tested, but resulted in poorer chromatographic performance.
- Higher formic acid concentrations increased MS response by producing narrower (more concentrated) peaks, without suppressing signal to a greater extent.
- This formic acid effect was more pronounced with a silica column than with a polymeric based RP chemistry.
- When building an LC/MS method for a protein mixture, tradeoffs between chromatographic and MS performance may be necessary.