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Polar And Non Polar Peptides Analyzed by LC-MS with an Aqueous Normal Phase ANP Method - AppNote

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Analyzing Polar and Non-Polar Peptides by LC-MS Using Aqueous Normal Phase (ANP) – Tips & Practical Suggestions

Peptide analysis can be particularly challenging due to the wide diversity in peptide polarity, structure, and ionization behavior. Conventional reversed-phase LC often struggles to retain very polar peptides, while highly hydrophobic peptides may require different chromatographic selectivities.

This article presents an effective solution for analyzing both polar and non-polar peptides using Aqueous Normal Phase (ANP) chromatography on Cogent Diamond Hydride™ columns.

Why ANP Works Exceptionally Well for Peptides

Broad Retention Capabilities

> Cogent Diamond Hydride™ columns offer unique selectivity by allowing polar peptides to be retained through aqueous normal phase mechanisms while still supporting hydrophobic interactions. This makes them exceptionally versatile for complex peptide mixtures.

Compatible with LC-MS Detection

> ANP mobile phases are fully LC-MS compatible, enabling high sensitivity for peptides that may vary widely in hydrophobicity.

Excellent Retention of a Wide Range of Peptides

> The published study demonstrates that Diamond Hydride™ columns can retain both extremely polar and moderately hydrophobic peptides, making the method well-suited for peptide discovery, impurity profiling, and complex mixture analysis.

Column Format & Method Adaptability

> Capillary ID Columns Used in the Publication
>
> The referenced study used capillary internal diameter columns, mainly for experimental convenience and sensitivity.

Works Equally Well with Standard LC-MS Column Diameters

The method is not limited to capillary formats. Standard analytical LC-MS column sizes can be used with similar selectivity and retention behavior, making the technique practical for routine laboratory workflows.

When to Choose ANP for Peptide Analysis

ANP on Diamond Hydride™ columns is particularly beneficial when:

- Your peptides are too polar for effective retention on reversed phase.
- Your mixture contains a wide polarity range, requiring a single method that handles both ends of the spectrum.
- You want high MS sensitivity without using ion-suppression additives.

This is often the case in peptide mapping, metabolomics, peptide degradant profiling, and small peptide drug analysis.

Supporting Method Resources

The article references an **AppNote** that includes specific method conditions such as:

- Mobile phase compositions for ANP
- Gradient recommendations
- MS-compatible buffer systems
- Flow rates and column temperature guidance

Access via the “Click HERE” link in the original article.

Conclusion

Aqueous Normal Phase chromatography using Cogent Diamond Hydride™ columns provides a robust, MS-compatible solution for analyzing polar and non-polar peptides in a single method. The stationary phase delivers exceptional retention range, making it a strong choice for diverse peptide mixtures and advanced LC-MS workflows.

COGENT HPLC Columns™

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