

Aqueous Normal Phase Reversed Phase and Normal Phase Definitions - HPLC Primer

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Aqueous Normal Phase (ANP) Defined & Compared to Reversed Phase and Normal Phase

Introduction

Aqueous Normal Phase (ANP) chromatography is a powerful retention mode that bridges characteristics of both traditional Normal Phase (ONP) and Reversed Phase (RP) separations.

It uses reversed-phase solvents—primarily water and acetonitrile—along with small amounts of acid or base, yet produces a retention pattern aligned with classic Normal Phase trends. This article defines ANP within the broader chromatographic framework and clarifies how its retention behavior differs from ONP and RP.

Understanding Normal Phase (ONP)

Normal Phase chromatography increases retention as the mobile phase becomes less polar, typically by decreasing water or another polar solvent. Highly non-polar mobile phases (e.g., 100% hexane) produce the longest retention times for polar analytes because analyte–stationary-phase interactions dominate when mobile-phase polarity is minimal.

Understanding Reversed Phase (RP)

In Reversed Phase chromatography, the opposite trend occurs: retention increases as the mobile phase becomes more polar. As water concentration rises, hydrophobic analytes interact more strongly with the non-polar stationary phase, generating longer retention times. This makes RP the most widely used mode for moderately non-polar or hydrophobic analytes.

What Defines Aqueous Normal Phase (ANP)

ANP chromatography creates a Normal Phase-like retention pattern using reversed-phase solvents such as acetonitrile and water, modified with small acid or base concentrations. Maximum retention typically occurs at very high organic levels, up to ~98% acetonitrile, with only ~2% aqueous content. As the aqueous portion increases, retention decreases, eventually reaching a minimum under low-ACN, high-water conditions. This behavior distinguishes ANP from both ONP and RP even though the solvent system resembles RP mobile phases.

Practical Interpretation

- In ANP, increased water reduces retention, mimicking Normal Phase behavior.

- ANP allows use of RP solvents, making it far easier to integrate with MS-compatible mobile phases compared to classical ONP.
- Because retention is strongest at high ACN, ANP is particularly effective for polar and ionic compounds that otherwise elute at or near void volume in standard RP methods.

Conclusion

Aqueous Normal Phase chromatography delivers Normal Phase retention behavior while maintaining the solvent compatibility, flexibility, and practicality of reversed-phase systems. By understanding the relationship between organic content and analyte retention, chromatographers can leverage ANP to retain polar analytes efficiently without reverting to traditional non-polar Normal Phase solvents.

Summary

Method	Column Type	Mobile Phase Polarity	Retention Increases with...	Targets
Normal Phase	Polar	Non-Polar	Less polar mobile phase	Polar Compounds
Reversed Phase	Non-Polar	Polar	More polar mobile phase	Non-Polar Compounds
ANP	Polar-like	Reversed Phase Solvents	Less polar mobile phase	Polar compounds

Want to learn more?

Check out these resources for deeper explanations:

- [Why Aqueous Normal Phase Methods are better than HILIC](#)
- Wikipedia: [Aqueous Normal Phase Chromatography](#)



Attachment: Using Aqueous Normal Phase White Paper 0.2 Mb [Download File](#)

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