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Aqueous Normal Phase ANP and How it is Useful for Polar Compound Separation - Tech Information

Date: 20-JUNE-2016 Last Updated: 3-NOVEMBER-2025

Aqueous Normal Phase (ANP) chromatography is a powerful and versatile HPLC technique that bridges the gap between Reversed Phase (RP) and Organic Normal Phase (ONP) chromatography. It's especially useful for separating polar compounds like acids, amines, and other water-soluble molecules.

🔍 What Is ANP Chromatography?

Definition: ANP is a unique HPLC retention mechanism that uses a mostly organic mobile phase with varying amounts of water depending on the analytes , and a slightly hydrophobic stationary phase. It's called "Aqueous Normal Phase" because:

- The mobile phase contains water (aqueous)
- The stationary phase is less polar than the mobile phase (normal phase behavior)

This setup allows for strong retention of polar analytes, which is often challenging in traditional reversed phase chromatography.

🔍 How Does ANP Compare to Other HPLC Modes?

Mode	Stationary Phase	Mobile Phase	Retention Mechanism	Best For
Normal Phase (NP)	Polar (e.g., silica, amino)	Non-polar (e.g., hexane + IPA)	Adsorption	Very polar compounds in organic solvents
Reversed Phase (RP)	Non-polar (e.g., C18, C8)	Polar (e.g., water + MeOH or ACN)	Partition	Non-polar to moderately polar compounds
HILIC	Very polar (e.g., bare silica, zwitterionic)	High organic (e.g., ACN) with low water	Partition into water layer on surface	Highly polar, water-soluble compounds
Aqueous Normal Phase (ANP)	Slightly hydrophobic (e.g., silica hydride)	Organic-rich (e.g., ACN) with low water	Adsorption for positive ions and ion displacement for negative ions	Polar compounds, acids, amines, zwitterions

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🧪 Key Differences Between ANP and HILIC

Feature	ANP	HILIC
Stationary Phase	Silica hydride (slightly hydrophobic)	Hydrophilic (e.g., bare silica)
Water Layer Formation	No	Yes

Retention Mechanism	Direct adsorption	Partitioning into water layer
Reproducibility	High (no water layer variability)	Can vary due to water layer thickness
MS Compatibility	Excellent	Excellent
Mobile Phase	High ACN with 2–5% water	High ACN with 5–20% water

💡 Why Use ANP for Polar Compounds?

- Better retention of polar compounds that elute too quickly in RP
- No need for high-pH mobile phases to retain basic compounds
- Compatible with MS detection using formic or acetic acid and ammonium salts
- No water layer formation, unlike HILIC, which improves reproducibility

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🧬 What Makes Silica Hydride Columns Special?

A NP is only possible on **silica hydride-based stationary phases**, which are different from traditional silica:

- Traditional silica has silanol (-Si-OH) groups → very hydrophilic
- Silica hydride has hydride (-Si-H) groups → slightly hydrophobic

This unique surface allows direct adsorption of polar analytes without forming a water layer, unlike HILIC columns. It also enables robust and reproducible retention.

⚙️ Mobile Phase Setup for ANP

- Typical solvents: Acetonitrile with 2–5% water
- Additives: Formic acid, acetic acid, ammonium formate, or ammonium acetate
- Gradient: Start with high organic (e.g., 95%-40% acetonitrile) and decrease to increase water content

✅ **Bonus:** You can switch between RP and ANP modes on the same column by simply adjusting the water content—no need to purge or change columns!

📚 Supporting Research

ANP retention using silica hydride based phases has been demonstrated in over 200 peer reviewed articles including a simple explanation:

> *“Hydride-based stationary phases: A rapidly evolving technology for the development of new bio-analytical methods”*
> J.J. Pesek et al., *Anal. Methods*, 6 (2014), 4496–4503.

🧪 Key Takeaways

- ANP is ideal for polar compound separations in HPLC especially in LCMS
- Uses silica hydride based columns and organic-rich mobile phases
- Offers robust, reproducible retention without the drawbacks of HILIC
- Ideal for mass spectrometry with common HPLC solvents

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