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Hydrophilic-Interaction Chromatography (HILIC) for LC-MS/MS Analysis of Monoamine Neurotransmitters using XBridge BEH Amide XP Columns

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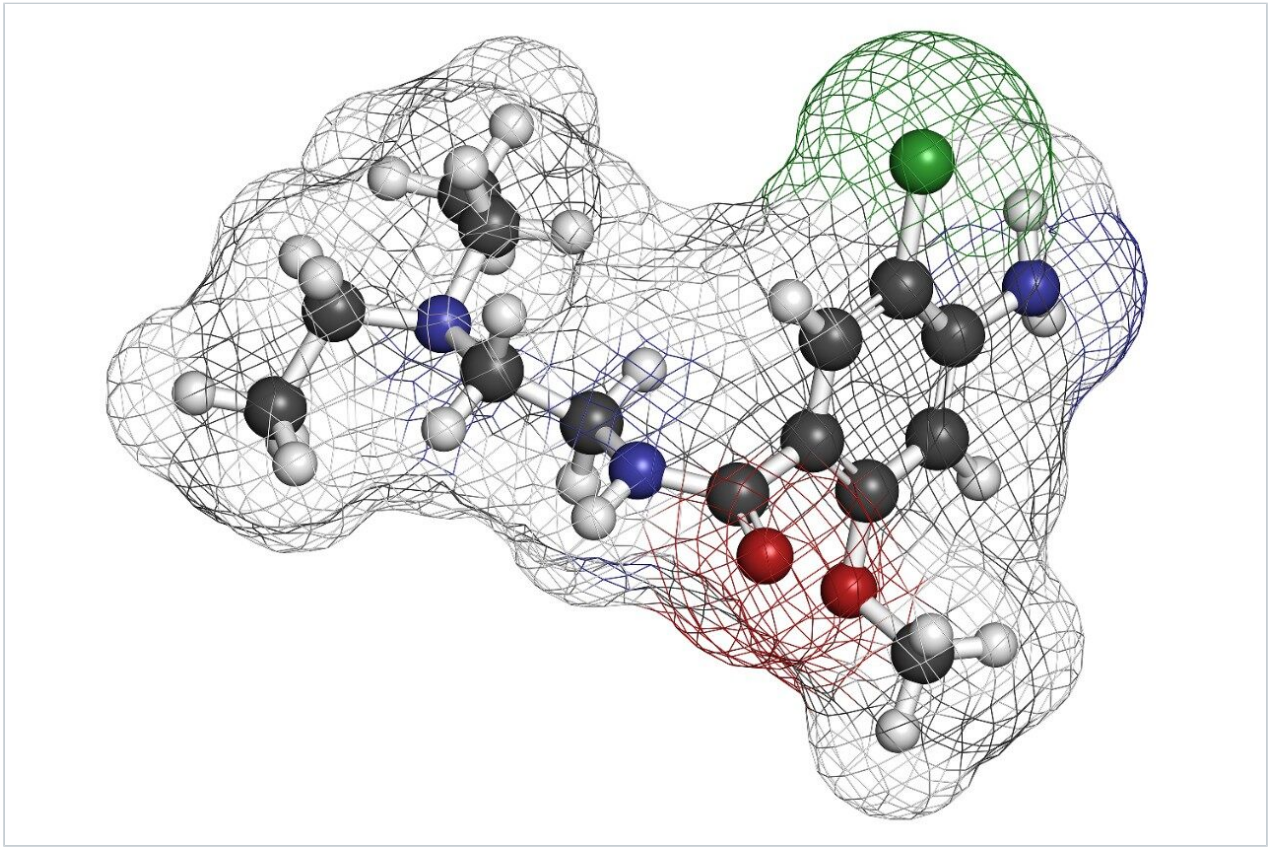

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Abstract

This aplication note presents the application of HILIC for the analysis of monoamine neurotransmitters using an XBridge BEH Amide XP column. This method achieves baseline separation for the most polar and challenging analytes in a short analysis time without the use of ion-pairing reagents that are often necessary in reversed-phase analysis of these compounds.

Benefits

- Retention and baseline resolution of monoamine neurotransmitters without the need for ion pairing reagents
- Short analysis time with low system backpressure allows the flexibility to substantially increase sample throughput
- Systematic method development strategies for HILIC
- 2.5 µm particle size provides the flexibility to operate on a variety of systems

Introduction

Hydrophilic-interaction chromatography (HILIC) is increasingly becoming a method of choice for the analysis of polar compounds [1-5]. One set of polar analytes that poses particular challenges are the monoamine neurotransmitters, dopamine (DA), serotonin (5-HT), epinephrine (EP), and norepinephrine (NE). These compounds play a significant role in many mood, movement, and other neurological disorders such as depression, anxiety, schizophrenia, and Parkinson's disease[6-8]. These neurotransmitters also play a critical role in the effects and toxicity of drugs of abuse[9-11].

This work presents the application of HILIC for the analysis of monoamine neurotransmitters using an XBridge BEH Amide XP column. Development of the successful chromatographic method depends upon the systematic optimization of organic mobile phase ionic strength to obtain the best peak shape and sensitivity. Choice of stationary phase and column temperature are also important. The resulting method achieves baseline separation for the most polar and challenging analytes in a short analysis time without the use of ion-pairing reagents that are often necessary in reversed-phase analysis of these compounds.

Experimental

Methods

Combined stock standards of dopamine, norepenephrine, epinephrine, serotonin, and N-methyl serotonin (NMS) were prepared in methanol containing 0.1% ascorbic acid and 2.5% 1N HCl to prevent oxidation. Working standards of 100 ng/mL DA, NE, EP, 5-HT, and 10 ng/mL NMS were prepared fresh each day in starting mobile phase conditions.

LC Conditions:

LC System:	ACQUITY UPLC
Column:	XBridge BEH Amide XP, 2.5 µm; 2.1 x 75 mm. P/N 186006090
Column Temp:	30 °C
Sample Temp:	5 °C
Mobile Phase A:	95:5 water:acetonitrile containing 100 mM NH_4HCOO, pH 3.0
Mobile Phase B:	85:15 acetonitrile:water containing 30 mM NH_4HCOO, pH 3.0
Needle Washes:	Strong and weak needle washes were both placed in MPB

Mass Spectrometry:

MS System:	Xevo TQ-S
Ionization mode:	ESI Positive
Acquisition mode:	MRM
Capillary Voltage:	2.0 kV
Cone voltage:	Compound specific (see Table 1)
Desolvation Gas:	900 L/hr
Cone Gas:	150 L/hr
Desolvation Temp:	350 °C
Source Temp:	100 °C

Analyte	MRM transition m/z	Cone Voltage	Collision Energy
NMS	191.1>160	30	15
5-HT	177.0>160	14	8
DA	154>137	18	8
EP	184>166	12	8
NE	152>107	30	14

Table 1. Mass spectral parameters used for analysis of monoamine neurotransmitters under HILIC conditions.

Data was acquired and analyzed using MassLynx Software (V4.1; SCN 810).

Initial mobile phase conditions were 100% MPB. The percentage of MPA was increased to 30% over 2.5 min. The percentage of MPB was returned to 100% over 0.1 minute and held there for 1.4 min. The total cycle time was 4.0 min. The injection volume was 20 µL.

Results and Discussion

Optimization of Mobile Phase Composition

Figure 1A shows the chromatography of monoamine neurotransmitters analyzed on the XBridge BEH Amide XP column. In order to achieve acceptable peak shape and resolution, it was necessary to carefully balance the ionic strength of MPB with solubility. 30 mM NH_4HCOO in MPB was required to achieve the chromatographic performance seen in Figure 1. The use of mobile phases with lesser ionic strength (10 and 20 mM) resulted in significant peak tailing and poor resolution between DA, EP, and NE, the most polar analytes, possibly due to secondary interactions with the stationary phase itself. Increasing the aqueous content of MPB to 15% ensured complete miscibility and prevented phase separation between water and acetonitrile that occurred at lower aqueous concentrations. Increasing the aqueous content alone resulted in a predictable decrease in retention for all compounds, but did not result in any improvements in peak shape or resolution between adjacent peaks. These changes are all consistent with the theory that increasing mobile phase ionic strength can disrupt secondary interactions with the stationary phase, resulting in improved chromatography.

Choice of Stationary Phase

The performance of the XBridge BEH Amide XP column detailed above was compared to the unbonded hybrid particle (XBridge BEH HILIC XP). Preliminary work with 10 mM ammonium formate in MPA and MPB had shown that the compounds in this study exhibited better separation and resolution on the Amide column vs. the XBridge HILIC column. Comparison of the two columns using the optimized conditions described above confirmed those initial results. Figure 1B shows chromatograms of monoamine standards analyzed on the XBridge BEH HILIC XP column. Clearly, retention of nearly all analytes is superior on the Amide column as is the resolution between adjacent peaks. The superior performance of the Amide column may be attributable to its polar functional group. In an acidic environment (pH 3.0), the polar nature of the amide functionality may be more effective at interacting with the aqueous portion of the mobile phase and forming the stagnant water layer required for HILIC chromatography.

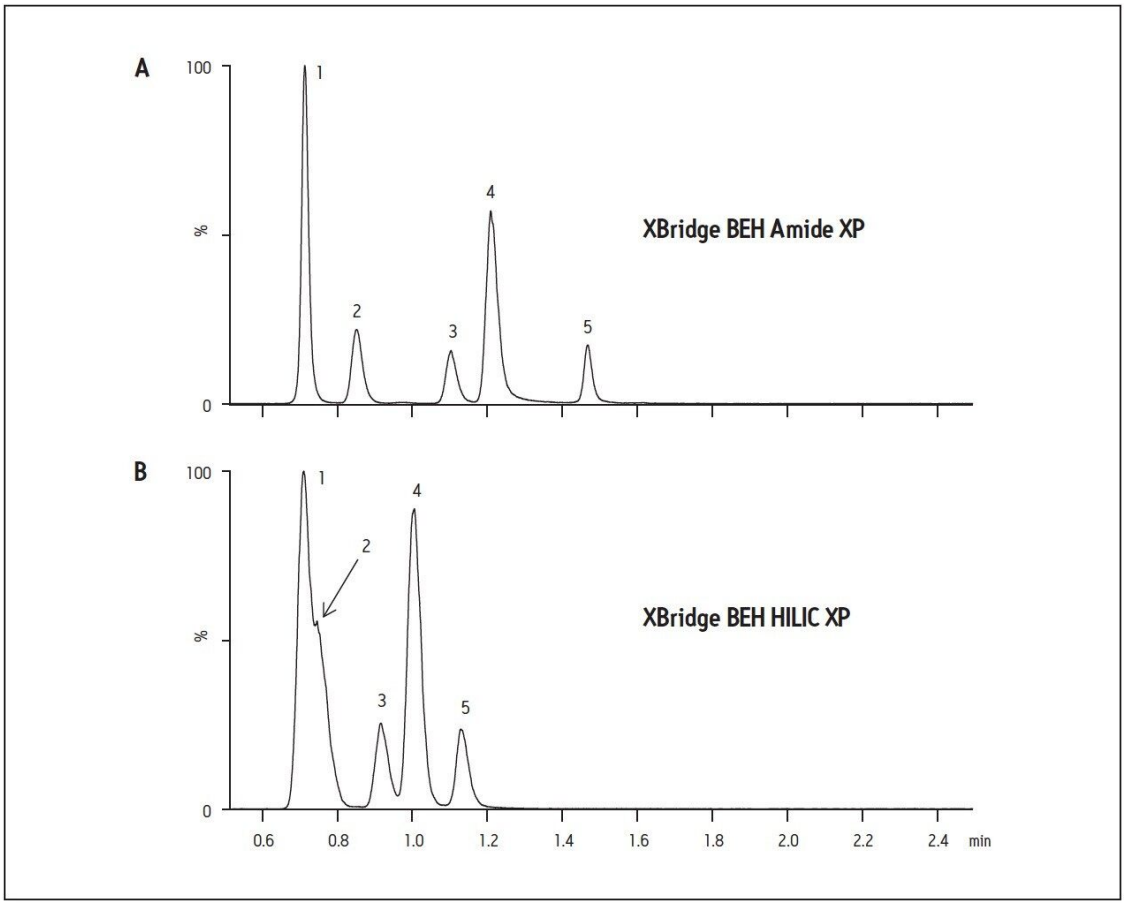

Figure 1: Chromatography of monoamine neutotransmitters on Waters BEH XBridge BEH Amide XP (A) and XBridge BEH HILIC XP (B) columns. Analyte key: 1-NMS, 2-5-HT, 3-DA, 4-EP, 5-NE.

Effect of Temperature on HILIC Chromatography

HILIC chromatography can be quite sensitive to temperature, and both decreases and increases in retention have been seen with temperature increases[12, 13]. Figure 2 shows chromatograms of monoamines run under the mobile phase conditions used in Figure 1A at different temperatures. In general, as temperature increased, the resolution between peaks decreased. At 40 °C, there is a decrease in the resolution between dopamine and epinephrine and at 60 °C, baseline separation has clearly been lost. Interestingly, when the column was cooled to 20 °C, there was a significant loss of peak shape for NMS. As this figure clearly shows, 30 °C provided the optimum balance of speed, resolution, and peak shape for all analytes.

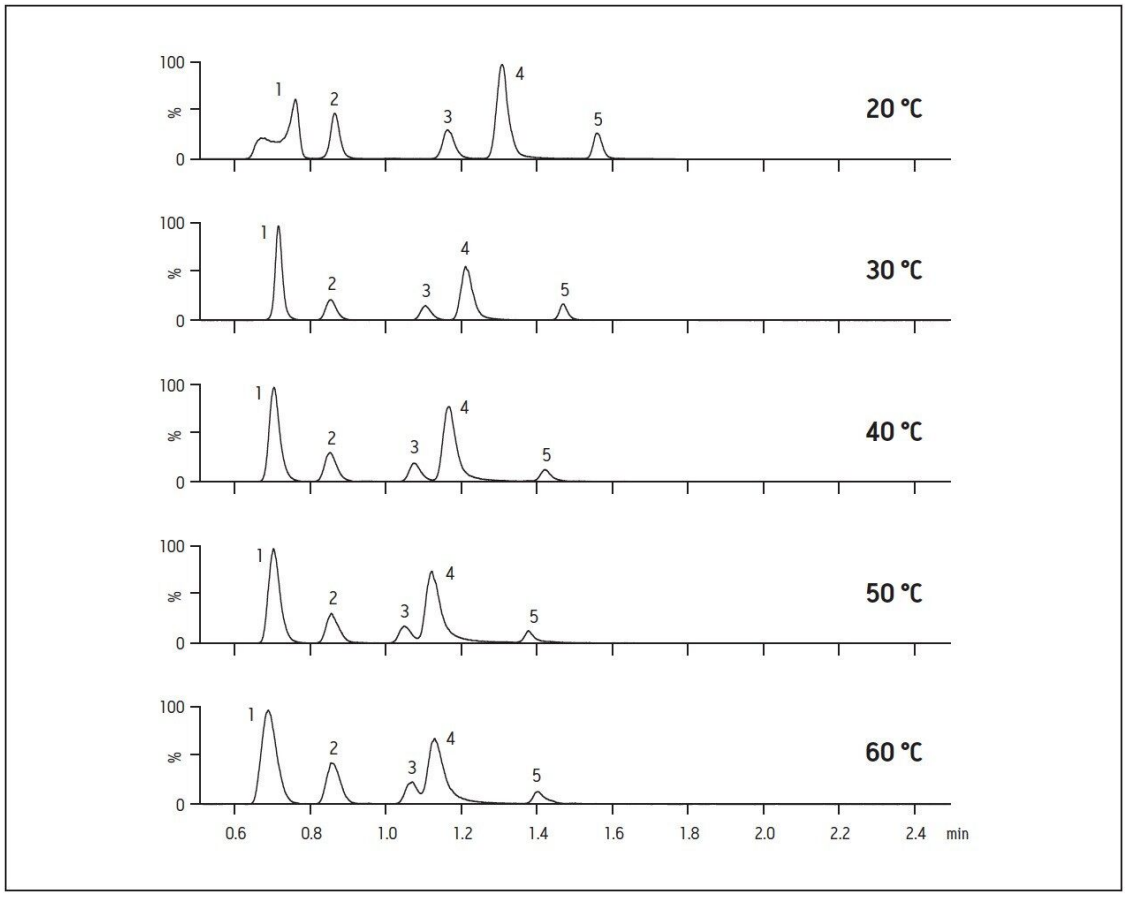

Figure 2: Effect of changes in column temperature on monoamine chromatography. Analyte key: 1-NMS, 2-5-HT, 3-DA, 4-EP, 5-NE.

Conclusion

The development of HILIC chromatography for the analysis of monoamine neurotransmitters using a 2.5 µm particle column is detailed. Optimizing the ionic strength of the organic mobile phase proved critical to maximizing chromatographic performance, but needed to be balanced with increases in aqueous content to ensure complete solubility. The superior performance of the XBridge BEH Amide XP column demonstrates the importance of stationary phase choice considerations during HILIC method development. Column temperature is also shown to be a crucial consideration for optimizing chromatography. These results show that retention, separation and resolution of even the most polar compounds (epinephrine and norepinephrine) can be readily achieved. The intermediate length of the column (75 mm) combined with the

relatively low backpressures characteristic of HILIC analyses should afford ample flexibility to adapt this method as needed.

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