

# LC-MS/MS Method for the Analysis of Choline and Acetylcholine using a Synchronis HILIC 1.7 $\mu\text{m}$ Column

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## Key Words

Synchronis HILIC, Acetylcholine, Choline

## Abstract

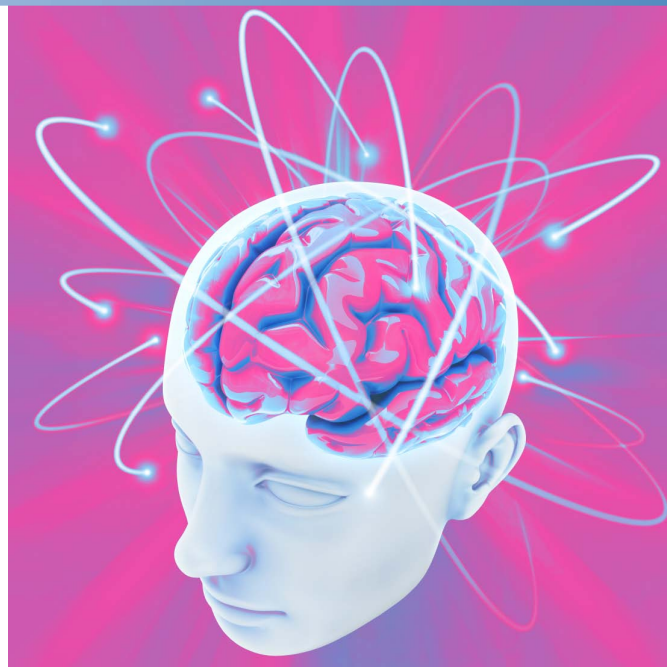
This application note demonstrates the use of the Thermo Scientific Synchronis HILIC 1.7  $\mu\text{m}$  column for the rapid LC-MS/MS analysis of acetylcholine and choline.

## Introduction

Choline is a water soluble essential nutrient that is produced in the body in small quantities. It also must be consumed in the diet to maintain healthy levels and one of the major food sources are eggs. Choline is a precursor of acetylcholine which is an important neurotransmitter involved in muscle control and memory.

One of the key goals for the chromatographer is to achieve a consistent, reproducible separation. The selection of a highly reproducible HPLC column is essential if this goal is to be attained. The Synchronis™ column range has been engineered to provide exceptional reproducibility due to its highly pure, high surface area silica, dense bonding and double endcapping, all controlled and characterized through the use of rigorous testing.

Choline is a small, highly polar quaternary amine and will not be retained using a C18 column. The zwitterionic nature of Synchronis HILIC makes it ideal for the retention of this class of compound. This application note demonstrates the successful analysis of acetylcholine and choline using a Synchronis HILIC 1.7  $\mu\text{m}$  column.



## Experimental Details

Consumables	Part Number
Fisher Scientific LC-MS grade water	W/0112/17
Fisher Scientific LC-MS grade ammonium formate	A115-50
Fisher Scientific LC-MS grade formic acid	A117-50
Fisher Scientific LC-MS grade acetonitrile	A/0638/17
Acetylcholine and choline purchased from Sigma Aldrich	
Thermo Scientific National Mass Spec Certified	MSCERT4000-34W
2 mL clear vial with blue bonded PTFE silicone cap	

## Sample Preparation

Sample contained 100 ng/mL of acetylcholine and choline in acetonitrile. The sample was dissolved in acetonitrile to ensure solvent compatibility with the HILIC method.

Separation Conditions	Part Number	
Instrumentation:	Thermo Scientific Accela 600	
Column:	Synchronis HILIC 1.7 $\mu$ m, 50 x 2.1 mm	97502-052130
Mobile phase:	ammonium formate, 15 mM, pH 3.5/ acetonitrile (10:90 v/v)	
Flow rate:	0.6 mL/min	
Column temperature:	30 °C	
Injection volume:	2 $\mu$ L	
Wash injection wash solvent:	20:80 (v/v) acetonitrile / water	
Strong injection wash solvent:	45:45:10 (v/v/v) acetonitrile / acetone / isopropanol	
Measured backpressure:	135 bar	

## MS Conditions

Instrumentation: Thermo Scientific TSQ Vantage

TSQ Vantage™ Conditions	
<b>Ionization conditions</b>	HESI
<b>Polarity</b>	Positive
<b>Spray voltage (V)</b>	3000
<b>Vaporizer temperature (°C)</b>	300
<b>Sheath gas pressure (mTorr)</b>	50
<b>Aux gas pressure (mTorr)</b>	30
<b>Capillary temp (°C)</b>	300
<b>Collision pressure (mTorr)</b>	1.5
<b>Cycle time(s)</b>	0.02
<b>Q1 (peak width)</b>	0.02
<b>Q3 (peak width)</b>	0.7

Compound Transition Details		
Compound	Acetylcholine	Choline
<b>Precursor ion (m/z)</b>	146.0	104.0
<b>Product ion (m/z)</b>	87.2	60.3
<b>Collision energy (eV)</b>	13	17
<b>S-lens (RF voltage)</b>	59	60

## Data Processing

Software: Thermo Scientific LC QUAN

## Results

The analysis was performed on a Synchronis HILIC 1.7  $\mu\text{m}$ , 50 x 2.1 mm column. As shown in Figure 1, acetylcholine is well retained ( $k' \sim 5$ ) and separated from choline in less than three minutes with excellent peak shape. Table 1 shows the results from six replicate injections and illustrates good reproducibility of retention time.

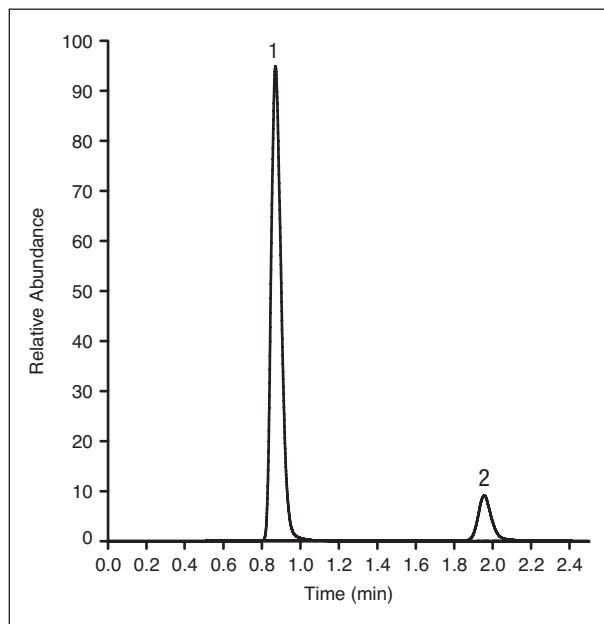


Figure 1. Chromatogram of acetylcholine (1) and choline (2) analyzed using a Synchronis HILIC 1.7  $\mu\text{m}$ , 50 x 2.1 mm column

Compound	Retention Time (mean)	Retention Time %RSD
Acetylcholine	0.87	0.1
Choline	1.96	0.1

Table 1. Precision data for acetylcholine and choline (calculated from 6 injections)

## Conclusion

Replicate injections of acetylcholine and choline showed that Synchronis HILIC 1.7  $\mu\text{m}$  produced stable and reproducible results with excellent peak shape. This demonstrates that Synchronis HILIC is an excellent choice of column for the rapid LC-MS/MS analysis of acetylcholine and choline.

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