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A Sensitive ESI-MS HILIC Method for the Analysis of Acetylcholine and Choline

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Hydrophilic interaction chromatography (HILIC) is a useful technique for the retention of polar analytes that offers a difference in selectivity compared to traditional reversed-phase (RP) chromatography. The highly volatile organic mobile phases used in HILIC provide increased electrospray ionization-mass spectrometry (ESI-MS) sensitivity. An Atlantis™ HILIC Silica column was used to develop a quantitative HILIC LC-MS method for the analysis of acetylcholine and choline. A limit of detection of 0.1 ng/mL was achieved for acetylcholine on a single quadrupole mass spectrometer.

Introduction

Hydrophilic interaction chromatography (HILIC) utilizes mobile phases that are highly volatile (> 80% organic) which is ideal for efficient desolvation and compound ionization, in effect enhancing signal response in mass spectrometry compared to traditional RP chromatographic methods. This enhancement effect will allow for low limits of detection. An Atlantis™ HILIC Silica column was used to develop a sensitive, quantitative HILIC-based LC-MS method for the analysis of acetylcholine and choline.

Experimental Conditions

Choline (Ch), acetylcholine (ACh) and choline- d_9 (Ch- d_9 , internal standard) were prepared in 75:25 acetonitrile:methanol with 0.2% formic acid. Separations were performed on an Atlantis™ HILIC Silica column (2.1 × 50 mm, 3 μ m) at ambient temperature. The mobile phase consisted of acetonitrile-water (86:14, v/v) containing 10 mM ammonium formate adjusted to pH 3.0. An isocratic separation was performed at a flow rate of 0.3 mL/min. The injection volume was 20 μ L. Mass spectrometer settings were:

Ionization: positive ion electrospray (ESI+)

Mode: SIR (m/z), ACh 146.2, Ch 103.9, Ch- d_9 113.1

Cone gas flow rate: 50 L/h

Desolvation gas flow: 700 L/h

Capillary voltage: 1.0 kV

Cone voltage: ACh 15 V, Ch 30 V, Ch- d_9 30 V

Extractor voltage 3.0 V

RF lens 0.3 V

Source temperature: 150 °C

Desolvation temperature 350 °C.

Data management and acquisition was performed using Empower™ Build 1154. Analysis was achieved on a Waters Alliance® HT 2795 Separations Module with Waters ZQ™ 2000 Mass Analyzer.

Results

Extracted ion chromatograms demonstrating the retention and selectivity of these analytes are shown in Figure 1. This highly sensitive method results in a limit of detection of 0.1 ng/mL (100 Fg/ μ L) for ACh on a single quadrupole mass spectrometer. Analyte concentrations were analyzed over the working range of 0.1–100 ng/mL to determine the limit of detection (LOD) and limit of quantitation (LOQ). Ch- d_9 was used as the internal standard and held at a constant 5 ng/mL. Data from the calibration curves generated are listed in Table I. The data was best fit using a weighted ($1/x$) linear regression yielding correlation coefficients of 0.9948 and 0.9993 for Ch and ACh, respectively. Due to a limitation of the linear range for Ch, the LOD and LOQ were determined to be 1.0 ng/mL and 2.5 ng/mL, respectively. Based on a signal-to-noise ratio of 3:1, the LOD for ACh was 0.1 ng/mL. Based on a signal-to-noise ratio of 10:1, the LOQ for ACh was 0.25 ng/mL.

Conclusions

The Atlantis™ HILIC Silica column offers a unique selectivity that retains and resolves Ch and ACh. This unique selectivity led to the development of a sensitive HILIC-ESI-MS method which allows for detection levels once only obtainable on a tandem MS system on a single quadrupole mass spectrometer.

Table I: Precision and accuracy of calibration standards

ACh (ng/mL)										
Nominal concentration	0.10	0.25	0.50	1.00	2.50	5.00	10.00	25.00	50.00	100.00
Calculated mean concentration	0.11	0.24	0.47	1.15	2.41	4.71	9.74	24.76	48.63	102.13
RSD (%)	4.88	4.59	2.93	3.26	1.98	1.93	2.45	1.66	1.82	1.05
RE (%)	8.65	-5.06	-5.32	15.38	-3.61	-5.88	-2.60	-0.95	-2.74	2.13
Ch (ng/ml)										
Nominal concentration	1.00	2.50	5.00	10.00	25.00	50.00	100.00			
Calculated mean concentration	1.37	2.43	4.17	8.29	23.47	49.50	104.24			
RSD (%)	0.79	2.22	1.44	0.97	0.53	1.63	1.33			
RE (%)	36.50	-2.63	-16.55	-17.07	-6.13	-1.00	-4.24			

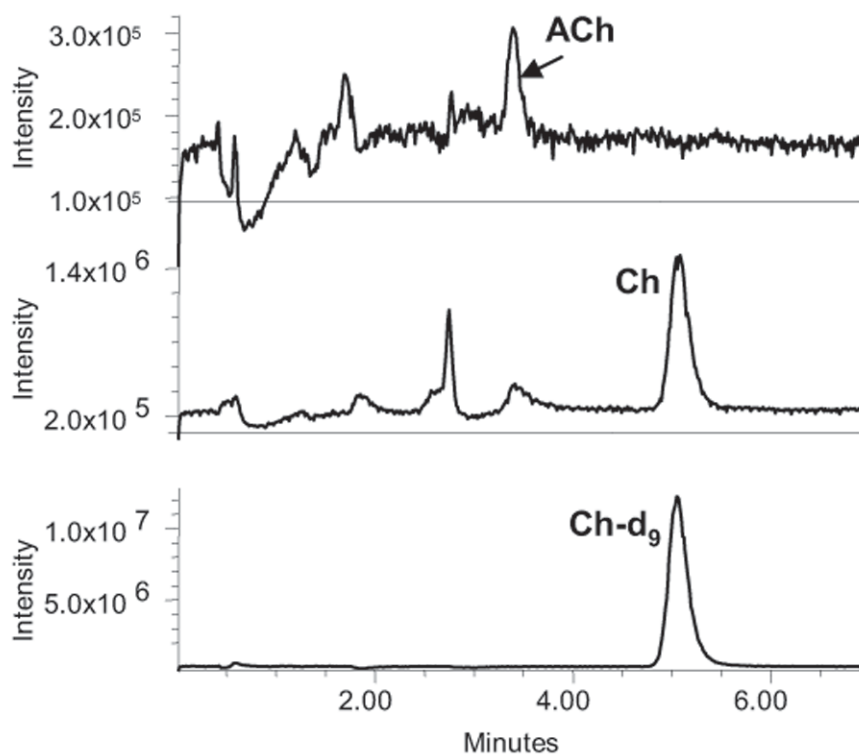


Figure 1: Low limits of detection achievable by a single quadrupole mass spectrometer. LC-MS SIR extracted ions (m/z) of ACh 146.2, Ch 103.9, and Ch- d_9 (I.S.) 113.1. Sample concentrations: ACh 0.1 ng/mL, Ch 1.0 ng/mL, and Ch- d_9 5 ng/mL.

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