Medical/Biological

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 with 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.

Experimental Conditions

Choline (Ch), acetylcholine (ACh) and choline-d₉ (Ch-d₉, 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 µm) 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 µL. Mass spectrometer settings were:

- Ionization: Positive ion electrospray (ESI+)
- Mode: SIR (m/z), ACh 146.2, Ch 103.9, Ch-d₉ 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-d₉ 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/µL) for ACh on a single quadrupole mass spectrometer. Analyte concentrations were analysed over the working range of 0.1–100 ng/mL to determine the limit of detection (LOD) and limit of quantitation (LOQ). Ch-d₉ was used as the internal standard and held at a constant 5 ng/mL. Data from the calibration curves.
generated are listed in Table 1. 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. Because of 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 that allows for detection levels once only obtainable on a tandem MS system on a single quadrupole mass spectrometer.

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