Isotopic Dilution Liquid Chromatography-Tandem Mass Spectrometry for the Determination of Acetylcholine in Cultured Cells

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Acetylcholine (ACh) is an essential messenger in neurotransmission in both the peripheral and central nervous system (CNS). It has an effect on alertness, memory and learning. Decreased levels of ACh are found in patients with Alzheimer's disease. In order to develop drugs against this disease, the ongoing processes in the neuronal cell, leading to a depletion of ACh have to be understood. Cell cultures are a common and useful system to investigate the molecular biology of tissues. To investigate the role of the signal cascades or the effect of potential drugs on the ACh synthesis in the cells, the ACh concentration in the cells has to be determined.

Therefore, a fast liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the analysis of acetylcholine (ACh) in cultured cells. D_4-Acetylcholine (D_4-ACh) was used as internal standard for calibration. ACh was extracted from the cell lysate with acetonitrile (ACN)/water (80/20, *v/v*) and the crude extract was analysed without further purification. Isocratic hydrophilic interaction chromatography (HILIC) with (10 mM) ammonium formate/ACN (35/75, *v/v*) as mobile phase was used for separation. ACh eluted within five minutes and was detected using electrospray MS/MS in the positive ion mode. The limit of detection was 1.5 fmol (0.3 nmol/L) ACh with a S/N ratio of 3:1.
The approach was used for the measurement of ACh in undifferentiated SN56 cells and the ACh content was determined to 1272±109 pmol/mg protein.