Supplementary Online Content


eMethods

This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods

Two replicates (capsule) from each dietary supplement sample were analyzed for their piracetam content. Powder from capsules was weighed in a 50mL conical tube and reconstituted in methanol to make a 100 mg powder/mL mixture. The samples were vortexed for one hour, centrifuged at 3000rpm for 10 minutes, and the supernate serially diluted to 100ng/mL in 10% acetonitrile. Internal standard (50ng/mL) was added and the sample was injected from the 10ug/mL, 1ug/mL and 100 ng/mL dilutions into the LC-QTOF/MS (Agilent LC 1260- QTOF/MS 6550, Agilent Technologies, Sta. Clara, CA) for analysis.

A 2.5uL diluted sample was injected in an Agilent Poroshell 120 C-18 column (2.1X 100mm, 2.7um) maintained at 55°C in each LC-QTOF/MS run. Chromatographic separation was achieved by gradient elution using LC-MS grade water with 0.05% formic acid and 5 mM ammonium formate as mobile phase A and acetonitrile with 0.05% formic acid as mobile phase B. The elution gradient employed was- 0-0.5 min= 5% B; 1.5 min= 30% B; 4.5 min= 70% B; 7.5 min= 100% B; 7.5-10 min= 100% B; and 10.01-12 min= 5% B. Eluates from the chromatographic column were ionized in the QTOF/MS in positive polarity operated in the following conditions: gas temperature at 225° C; sheath gas temperature at 350° C; drying gas flow at 14 L/min; sheath gas flow at 11 L/min; nebulizer pressure at 14 psi; voltage cap at 3000 V; and, nozzle voltage at 500 V. Data acquisition was run at 2 GHz in extended dynamic range mode.

The data obtained from the LC-QTOF/MS run was analyzed using Agilent MassHunter Qualitative Analysis software v B.6.0 (Agilent Technologies, Sta Clara, CA). All samples were analyzed for piracetam using the following criteria: mass error ≤ 10ppm; retention time ≤ 0.15 min; and, target score ≤ 70 (indication of isotopic pattern match) for peaks that did not exhibit detector saturation. Quantification of piracetam was done by isotope dilution method using an eight-point calibration curve, a reference standard of piracetam (Toronto Research Chemicals, Ontario, Canada), and hydromorphone-d6 (Cerilliant Corporation, Round Rock, TX) as internal standard. Piracetam was added to a comprehensive drug analysis panel consisting of 931 target analytes. The panel uses a set of ten internal standards for quantifying all analytes. Hydromorphone-d6 has the closest retention time to piracetam among the internal standards and was chosen as internal standard for quantitation. Agilent MassHunter Quantitative Analysis (Agilent Technologies, Sta Clara, CA) was used for data analysis. Each sample was run three times to obtain the mean content value for each replicate sample.