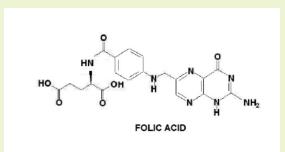
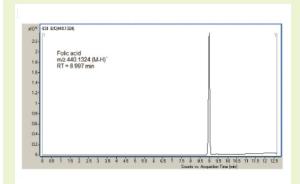


Folic Acid by LCMS

With Minimized Sample Prep & Increased Sensitivity





Notes: Deficiency of folic acid (vitamin B9) is associated with several chronic diseases (for example macrocytic anaemia, psychiatric disorders, neutral tube defects in newborns, cancer, Alzheimer's disease and cardiovascular disease in adults (1); thus accurate assessment of folate status has become important in the clinical setting, in epidemiological studies in clinical and research laboratories. FA cannot be synthesized by mammalian cells and therefore must be supplied with diet. It is very important to establish highly sensitive methods for the quantitative determination of trace amounts of this vitamin.

[1]. B.C. Nelson, K.E. Sharpless, L.C. Sander; J. Chromatogr. A, 1135 (2006) 203-211

Method Conditions

Column: Cogent Diamond Hydride™, 4µm, 100Å

Catalog No.: 70000-15P-2 **Dimensions:** 2.1 x 150 mm

Solvents: A: DI $\rm H_2O/10~mM$ ammonium acetate B: 90% acetonitrile/ 10% DI $\rm H_2O/10~mM$

ammonium acetate. pH = 7 adjusted using ammonia

 Gradient:
 time (min.)
 %B

 0
 100

 5
 90

 9
 50

 10
 100

Post Time: 5 min

Flow rate: 0.4 mL/min

Detection: ESI - neg - Agilent 6210 MSD TOF mass spectrometer

Sample: Sample stock solution was made in Solvent A at a concentration of 2.0 mg/L. Sample for analysis was made by diluting the stock with Solvent A, to which 0.1% ammonia was added. Sample final concentration was 0.2 mg/L. Please note that folic acid sample was prepared in solvent A only as opposite to samples of other acids which are prepared in 50% mixture of solvents A and B. When the sample of folic acid was prepared in 50%A + 50%B the MS signal was lower.

Discussion

A sensitive and reliable method was developed for the determination of folic acid (FA) and can be used in many applications including complex sample matrices without the solid-phase extraction cleanup. Since the method uses LC-MS (negative-ion) it provides fantastic sensitivity as well as great selectivity. The use of chelating agents or high concentration of salts in the mobile phase or ion pair reagents (non MS compatible) are not required as in older methods for FA analysis. The LOQ is dramatically better than existing methods with UV detection. When this methodology is used very fast equilibration between gradients is observed as well. Excellent precision was achieved using the presented method. Twenty consecutive injections resulted in %RSD = 0.26.