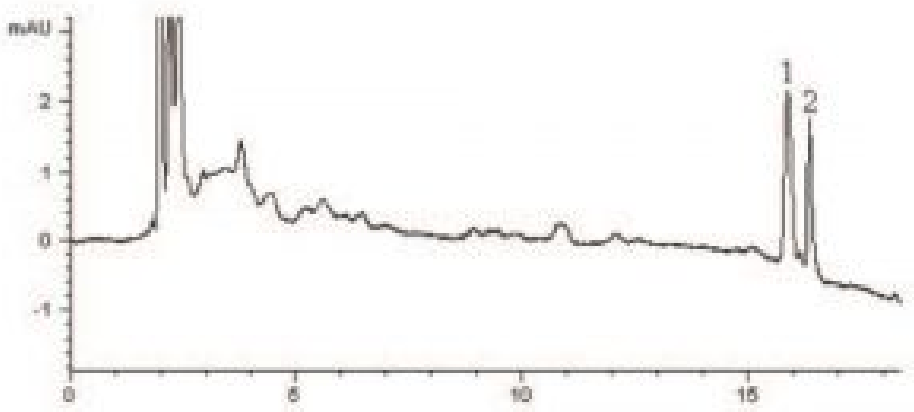
Folic Acid Content in Fortified Cereals Analysis by HPLC – AppNote

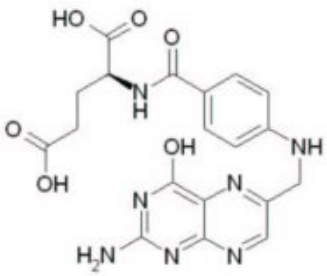
Online Cleanup of Sample or Matrix Components

This application note demonstrates an effective means of separating and resolving Folic Acid from undesired matrix components in cereal extracts without the use of SPE or other sample cleanup techniques. SPE is normally performed to remove compounds from the matrix which would co-elute with Folic Acid before injection but by performing the separation in this Method, this step can be virtually omitted.

Many of the matrix components in this cereal are less polar than Folic Acid and therefore elute earlier in the HPLC run. In this manner, the separation can act as an online sample cleanup and eliminate chances of losing any Folic Acid during this step. This helps to provide better reproducibility.

The precision of this method is clearly demonstrated by the low %RSD (0.1% and below) of the Folic Acid retention times. The calibration curve showed good linearity (R2 = 0.9997).





Peaks:

1. Folic Acid
2. Methotrexate: Internal Standard

Method Conditions

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

Catalog No.: 70000-7.5P

Dimensions: 4.6 x 75mm

Mobile Phase:

A: DI Water + 10mM Ammonium Formate

B: 90% Acetonitrile / 10 % DI Water / 10mM Ammonium Formate

Gradient:

Time *(minutes)*	%B
0	100
10	90
19	50
20	100

Post Time: 5 minutes

Flow rate: 0.5mL / minute

Detection: UV @ 284nm

Injection vol.: 1µL

Sample Preparation: Fortified whole wheat flour-based cereals were ground, dispensed in DI Water + 10mM Ammonium Formate + 0.05% (w/v) Sodium L-Ascorbate + 12mM NH_3. Next the sample was centrifuged at 10,000G. The supernatant was then collected and filtered through a 0.45µm Nylon Syringe Filter prior to HPLC-UV injections. (MicroSolv Technology Corp.)

Note: *Folic Acid (Pteroyl-L-Monoglutamic Acid) is a member of a biologically important class of compounds known as Folates and is added to many foods and beverages by what is referred to as fortification. To verify that the correct amount of Folic Acid has been added to fortified products, reliable analytical methods are needed for its quantitation in situ. Folate metabolites play a vital role in nutrition. Deficiency of Folates in the diet leads to an accumulation of Homocysteine [1]. Elevated levels of Homocysteine, in turn, have been shown to inhibit purine biosynthesis [2].*

[1] C. M. Ulrich, M. C. Reed, H. F. Nijhout, Nutr. Rev. 66 (2008) S27–S30.

[2] Y. Fujita, E. Ukena, H. Iefuji, Y. Giga-Hama, K. Takegawa, Microbiology. 152 (2006) 397.

Attachment

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