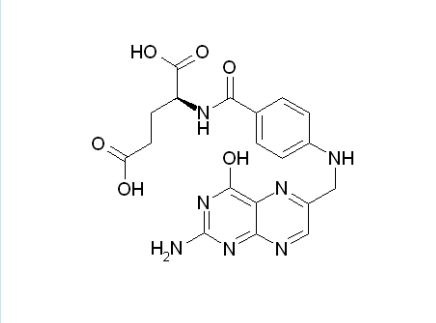
APPLICATION NOTES

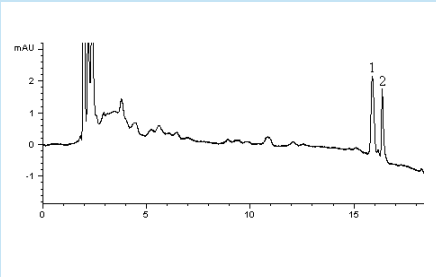
Cogent™ Diamond-Hydride

HPLC
ANP

Folic acid content in fortified cereals

Online Cleanup of Sample or Matrix Components





Notes: 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.

Method Conditions

Column: Cogent Diamond Hydride™ 4µm, 100Å.
Catalog No.: 70000-7.5P
Dimensions: 4.6x75mm
Solvents: A: DI water + 10 mM ammonium formate
B: 90% acetonitrile/10% DI water/10 mM ammonium formate
Both solutions were vacuum filtered through a 0.45 µm nylon filter (MicroSolv Technology Corp).

Gradient:

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

Post Tme: 5 min.
Flow Rate: 0.5 mL/min.
Samples: Fortified whole wheat flour-based cereals.
Ground, dispensed in DI H2O + 10 mM ammonium formate + 0.05% (w/v) sodium L-ascorbate + 12 mM NH3. Next sample was centrifuged at 10,000 g. The supernatant was then collected and filtered through a 0.45 µm nylon membrane HPLC filter prior to HPLC-UV injections. (MicroSolv Technology Corp.)
Peaks: 1. Folic acid
2. Methotrexate: internal standard
Detection: UV 284 nm

Discussion

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 shown above, 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 (R^2 = 0.9997).

Results obtained by this method agree with the amount of the folic acid reported by the manufacturer. Methotrexate was used as an internal standard for the developed method.

Cat. No.	Description
70000-7.5P	Cogent Diamond Hydride™ HPLC Column, 100A, 4µm, 4.6mm x 75mm

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