Instruction of the Clean-up Process using *B-TeZ IAC Folic Acid 3ml*

Fast and Accurate Content Determination of Folic Acid in Vitamin Tablets, Liquid Vitamin Preparations, Cell Culture Extracts etc. by Combination of Immunoaffinity Chromatography and HPLC-UV

Principle:
Many methods of Folic Acid determination based on HPLC-UV (High Performance Liquid Chromotography-Ultraviolet) or HPLC-FLD (High Performance Liquid Chromatography with Postcolumn Fluorescence Derivatization) detection show low selectivity if problematic matrices are applied.

This method of determination of Folic Acid combines the high selectivity of immunoaffinity columns (compared to other separation materials (e.g. SAX)) with its potential to concentrate elute through use of purification by a HPLC column.

Sample Preparation:
Folic Acid samples are to be extracted and analyzed with the method of E.S. Osseyi et al. [E.S. Osseyi, R.L. Wehling, J.A. Albrecht *J. Chromatogr*. A 1998; 826:235-240], e.g. vitamin tablets, liquid vitamin preparations, cell culture extracts. Example: 2g vitamin containing tablets are dissolved in 50ml PBS. The resulting extract may be filtered through a 0.45µm membrane filter.

Enrichment Step IAC:
10ml extract (containing the quantity of folic acid from a 0.4g sample if above-mentioned sample preparation is followed) is diluted with a total volume of 30ml PBS and then applied in a reservoir on top of the *B-TeZ IAC Folic Acid 3ml* column. The optimal flow rate through the gel is between 1 to 3 ml/min.

Wash:
After whole sample has passed through the gel the latter is washed with 5ml of PBS. Remaining liquids in the gel are removed by applying either pressure from top of the column or pressure from the bottom.

Elution:
The sample reservoir on top of the *B-TeZ IAC Folic Acid 3ml* column is removed, and an appropriate vial is placed below the affinity column. The bounded folic acid is eluted by using a total volume of 3ml of methanol-phosphoric acid solution (99.8/0.2 v/v). The elution process is performed in two steps. First, an amount of 1ml methanol is applied. Once this amount has passed through the column, there should be a waiting time of 30 seconds. After that, the second portion of 2ml of methanol is eluted through the column. The flow rate should lie below 3ml/min. The remaining methanolic solutions should be eluted by application of slight under- or overpressure. All methanolic fractions are unified to give the column elute.

The column elute may be injected into the HPLC directly or, if concentrations are very low, concentrated by evaporation at 50°C for 1h (e.g. using VLM evaporator), re-dissolved in HPLC solvent and finally injected into the system. For the latter case, please see the sample calculation in which the sample concentrate is re-dissolved in 1.0ml HPLC solvent.

Analytical Method:
- Machine: Shimadzu
- Column: Trentec Reprosil-Pur RP C18 120 ODS3 5µm 125x3,0mm with guard column
- Mobile Phase B: 0.03M potassium phosphate, pH 2.2-methanol (80/20 v/v)
- Gradient: 0.01min B 100%; 25min B 100% (isocratic)
- Flow Rate: 0.5ml/min
- Time of Analysis:25min
- Injector Volume: 100µl
- Detection: λ ABS [nm]: 280nm.

Characteristics:
The linear range is 20ng to 500ng Folic Acid, per injection (R2=0.9946).The limit of detection is 3 ng of folic acid per injection (3 times of signal/noise ratio). If the given dilution steps are followed, the folic acid contents of 0.5 to 12.5µg/g lie within the linear working range of the method. The lower limit of detection is 75ng/g of folic acid in the sample. The lower limit of detection may be reduced by enhancement of folic acid in the sample.

Recovery rates are >85% when folic acid in buffer mixtures is analysed in the range of 0.1 to 5µg per IAC.

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Example Sample Calculation:

2g Sample / 50ml Extraction Solvent	x	10ml Extract / 1ml	x	0.1ml injector volume	=	0.04g Sample Equivalents

# µg injected Folic Acid / Sample Equivalents [g]	=	µg/g Folic Acid

Buffer and Chemicals:

Acidic Elution Solvent:
methanol-phosphoric acid (99.8/0.2 v/v)

Dissolve 200µl phosphoric acid (95%) in 100ml methanol

HPLC-Solvent
0.03M potassium phosphate, pH 2.2-methanol (80/20 v/v)

Dissolve 4.1g KH_2PO_4 in 800ml deionized water. Adjust to pH 2.2 with phosphoric acid (95%). Add 200ml methanol. Degass with helium.

Chemicals:
- methanol, HPLC grade
- deionized water
- dipotassium hydrogenphosphate, >98%
- potassium dihydrogenphosphate, >98%
- sodium chloride
- phosphoric acid, 95%

Evaporation:
- nitrogen gas 5.0 [Air Liquide M55763810] (to evaporate IAC-eluate)

Consumables:
- ***B-TeZ IAC Folic Acid 3ml*** column [BTFS319005]

Standard:
- Folic Acid (98%) [Sigma F-7876]

Apparatus:
- HPLC; Shimadzu; pump: LC-6A (2 pieces); autosampler: SIL 6B; fluorescence detector: RF-10AXL; data handling: CLASS LC10
- Evaporator (with tripod) [VLM EVA EC1-S]
- Vakuum SPE Manifold (BAKER spe-24G Column Processor – process up to 24 samples) [J.T. Baker 7208]

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