EASI-EXTRACT® FOLIC ACID

Product Code: P81 / P81B

Immunoaffinity columns for use in conjunction with HPLC or LC-MS/MS.
For in vitro use only.

P81/V19/04.08.21

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R-BIOPHARM
RHÔNE LTD

Contents

Test Principle

The procedure is based on monoclonal antibody technology, which makes the test highly specific, sensitive, rapid and simple to perform.

The columns contain a gel suspension of monoclonal antibody specific to the vitamin of interest. Following extraction of the vitamin the sample extract is diluted with buffer, centrifuged and the supernatant filtered before being passed slowly through the immunoaffinity column. Any vitamin which is present in the sample is retained by the antibody within the gel suspension. The column is washed to remove unbound material and the vitamin is then released from the column following elution with solvent. The eluate is collected prior to analysis by HPLC or LC-MS/MS.

The total extraction and clean-up time takes approximately 3 hours to perform. The result is improved clean-up and concentration of the vitamin from food and feed samples giving a much cleaner chromatogram and therefore providing more accurate and sensitive detection. The columns also have the added advantage that they can be automated for large scale analysis of samples.

Reagents Not Provided

- Distilled / Deionised Water (suitable for use with HPLC, e.g. MilliQ)
- Solvents (Acetonitrile)
- Folic Acid Standard (Please refer to Preparation of Standards section)
- Sodium Hydroxide
- Sodium L-ascorbate
- Sodium Phosphate Monobasic
- Sodium Phosphate Dibasic Heptahydrate
- Trifluoroacetic Acid (TFA)
- Pancreatin 1xUSP*

* Please note that it is advised to check all enzymes for natural vitamin content prior to analysis as they may contain traces of folic acid.

Accessory Products

- Whatman S&S 597½ Filter Paper
- Immunoaffinity Column Rack (CR1)*
- Immunoaffinity Column Accessory Pack (AP01)*

* Available from R-Biopharm. Please contact your local R-Biopharm distributor for further information.

Recommended Methods and Application Notes

Methods are available for all matrices covered by legislation as well as additional commodities. Deviation from the methods described in our Instructions For Use and Application Notes may not achieve optimum results. Please contact your local R-Biopharm distributor for further information.

Hazards

Suitable protective clothing, including gloves, safety glasses and lab coats should be worn throughout the analysis.

Flammable solvents should be stored in an explosion-proof cabinet. Use a chemical hood and protective equipment as applicable.

Contact your local R-Biopharm distributor for a Material Safety Data Sheet for further information if required.

Decontamination

Glassware should be thoroughly washed and rinsed before use to avoid cross contamination.

Storage & Shelf Life

The columns expire 18 months from date of manufacture if stored at 2 - 8 °C or 12 months from date of manufacture if stored at 21 - 25 °C. Do not freeze.

Ensure the column has not dried out and contains buffer above the gel. It is important to note the antibody included in the immunoaffinity column can be denatured by extreme temperature or pH change.

Sampling

A representative sample should be obtained. It is recommended that the representative sample is finely ground and a portion (1 - 10 g dependent on method used) of this is removed and extracted.

Sensitivity

The sensitivity is dependent on the final detection system employed by the analyst. However the test sensitivity may be improved if required by increasing the volume of sample passed through the immunoaffinity column.

For optimal column performance, taking into account the LOQ of a typical HPLC system, aim to load sample containing a quantity of 0.1 - 0.5 µg of folic acid onto the column. Do not exceed a quantity of 0.45 µg as this is close to the capacity.

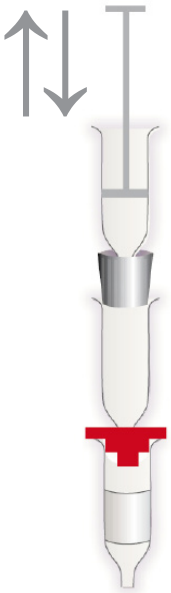
Recoveries

If an analyst wishes to account for losses during extraction it is recommended a spiked sample of the same commodity type as the material being tested is analysed following the complete procedure as a reference standard. The recoveries obtained with the spiked sample can be used to correct the results obtained with the test sample.

Column Preparation

Immunoaffinity columns should be at ambient temperature before use. Remove the cap from the top of the column and discard. Firmly attach the column to a glass syringe barrel using an adapter and place in an immunoaffinity column rack or clamp stand.

Backflushing



Backflushing is carried out to increase the time the solvent is in contact with the antibody within the gel suspension ensuring that all of the toxin is eluted. Backflush by gently raising and lowering the syringe plunger during passage of the solvent through the column. This process will reverse the direction of flow of the eluant. This should be repeated 3 times.

Preparation of 0.1 M Sodium Phosphate Buffer

The buffer can be kept for 5 days if stored at room temperature.

1. Weigh 4.68 g of monobasic sodium phosphate (anhydrous) and 16.37 g of sodium phosphate dibasic heptahydrate into a flask.
2. Make up to 1 L with water.
3. Check that the pH is 7.0.

Preparation of 10 % Sodium Ascorbate Solution

The solution should be prepared fresh on day of analysis.

1. Weigh 10 g of sodium l-ascorbate into a flask.
2. Add 100 ml of water.

Preparation of Mobile Phase Solution A (Water Containing 0.1 % TFA)

The solution should be prepared fresh on day of analysis.

1. Add 1 L of water to a flask.
2. Remove 1 ml to waste.
3. Add 1 ml of trifluoroacetic acid (TFA).

Preparation of Elution Solution (30 % Acetonitrile Containing 0.2 % TFA)

The solution can be kept for 2 days if stored at room temperature.

1. Add 100 ml of water to a flask.
2. Remove 200 µl to waste.
3. Add 200 µl of trifluoroacetic acid (TFA).
4. Remove 30 ml to waste.
5. Add 30 ml of 100 % acetonitrile.

Sample Preparation

• Infant Formula, Ready-to-Use Infant Formula and Liquid Milk

1. Weigh 5 - 10 g of sample into a 100 ml amber glass screw cap bottle.
2. Add 50 ml of 0.1 M sodium phosphate buffer (pH 7.0).
3. Place on a magnetic stirrer and add 4 g of pancreatin. Leave stirring for 10 minutes.
4. Add 6 ml of 10 % sodium ascorbate and leave sample to stir for a further 5 minutes.
5. Incubate the sample in a shaking water bath at 37 °C for 2 hours.
6. Transfer the sample to a second shaking water bath and incubate at 100 °C for 20 minutes. Remove the sample and allow to cool to room temperature.
7. Transfer the extract into a 100 ml amber volumetric flask and fill to the mark with 0.1 M sodium phosphate buffer.
8. Centrifuge the extract at 4,000 rpm for 10 minutes before filtering through a Whatman S&S 597½ filter paper.
9. Dependent on the commodity being analysed pass the appropriate volume of filtrate through the column according to the table below. Pass the filtrate through the column at a flow rate of 2 ml per minute (or the sample can be allowed to pass through the column by gravity if preferred). A slow, steady flow rate is essential for the capture of the vitamin by the antibody.

Commodity	Volume of Filtrate
Infant formula, dietetic milk powder	2 - 10 ml
Infant food formula, soya liquid milk	15 ml

10. Wash the column by passing 10 ml of water through at a flow rate of approximately 5 ml per minute. Pass air through the column to remove residual liquid.
11. Elute the vitamin from the column at a flow rate of 1 drop per second using 1 ml of elution solution and collect in an amber glass vial. Backflushing is recommended. Please refer to the Backflushing section for further information.
12. Following elution pass 1 ml of water through the column and collect in the same vial to give a 2 ml total volume.
13. Inject 100 µl onto the HPLC system.

Sample Preparation

• Cereal and Energy Bars

1. Dependent on the commodity being analysed weigh the appropriate amount of ground sample into a 100 ml amber glass screw cap bottle.

Commodity	Volume of Ground Sample
Cereal	1 - 10 g
Energy Bars	1.5 - 2 g

2. Add 50 ml of 0.1 M sodium phosphate buffer (pH 7.0).

3. Place on a magnetic stirrer and add 4 g of pancreatin. Leave stirring for 10 minutes.

4. Add 6 ml of 10 % sodium ascorbate and leave sample to stir for a further 5 minutes.

5. Incubate the sample in a shaking water bath at 37 °C for 2 hours.

6. Transfer the sample to a second shaking water bath and incubate at 100 °C for 20 minutes. Remove the sample and allow to cool to room temperature.

7. Transfer the extract into a 100 ml amber volumetric flask and fill to the mark with 0.1 M sodium phosphate buffer.

8. Centrifuge the extract at 4,000 rpm for 10 minutes before filtering through a Whatman S&S 597½ filter paper.

9. Dependent on the commodity being analysed pass the appropriate volume of filtrate through the column according to the table below. Pass the filtrate through the column at a flow rate of 2 ml per minute (or the sample can be allowed to pass through the column by gravity if preferred). A slow, steady flow rate is essential for the capture of the vitamin by the antibody.

Commodity	Volume of Filtrate
Cereal	5 - 10 ml
Energy Bars	10 ml

10. Wash the column by passing 10 ml of water through at a flow rate of approximately 5 ml per minute. Pass air through the column to remove residual liquid.

11. Elute the vitamin from the column at a flow rate of 1 drop per second using 1 ml of elution solution and collect in an amber glass vial. Backflushing is recommended. Please refer to the Backflushing section for further information.

12. Following elution pass 1 ml of water through the column and collect in the same vial to give a 2 ml total volume.

13. Inject 100 µl onto the HPLC system.

Preparation of Standards

Powdered folic acid can be purchased. The powder is dissolved to give a concentration of 200 µg/ml. Leave overnight at 2 - 8 °C to give a stock solution. All standards should be prepared in amber glassware.

Preparation of Diluent Solution (15 % Acetonitrile Containing 0.1 % TFA)

The solution can be kept for 2 days if stored at room temperature.

1. Add 100 ml of water to a flask.
2. Remove 100 µl to waste.
3. Add 100 µl of trifluoroacetic acid (TFA).
4. Remove 15 ml to waste.
5. Add 15 ml of 100 % acetonitrile.

Calibration Curve

It is recommended to run at least a 3 - 6 point calibration curve. In constructing a suitable curve the levels of the calibration standards should bracket or include the range of expected results. The diluted standard solutions should be prepared fresh on the day of analysis and used within a 24 hour period.

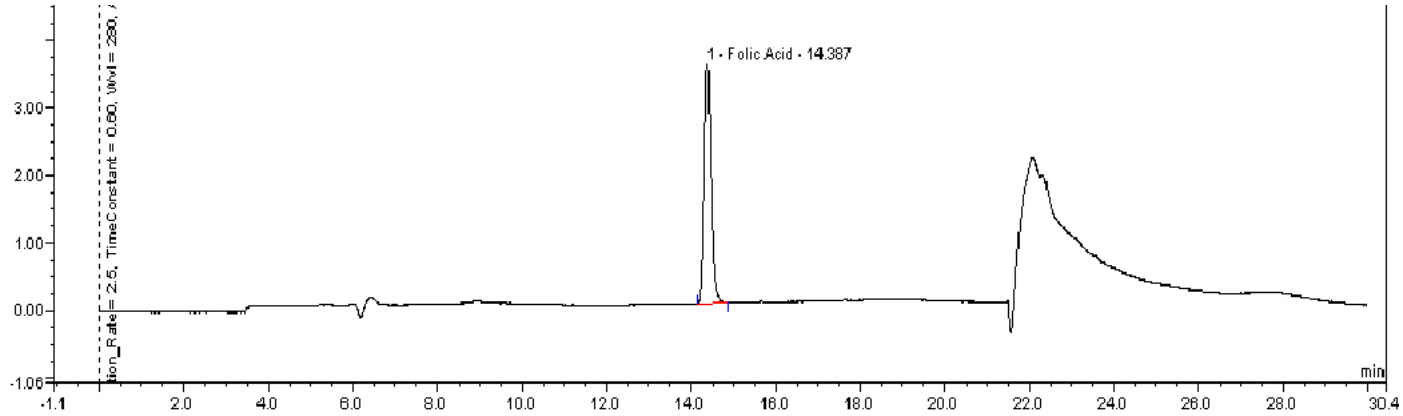
Example of how to prepare a five point calibration curve (can be modified according to expected vitamin content):

1. Measure 10 ml of water into an amber vial and remove 0.5 ml to waste.
2. Add 0.5 ml of 200 µg/ml stock solution to give a 10 µg/ml folic acid solution.
3. Standard 5: Take 10 ml of diluent solution and remove 1 ml to waste. Add 1 ml of 10 µg/ml (equivalent to 1 µg/ml).
4. Standard 4: Take 1 ml at 1 µg/ml and add 1 ml of diluent solution (equivalent to 0.5 µg/ml).
5. Standard 3: Take 1 ml of 0.5 µg/ml and add 1 ml of diluent solution (equivalent to 0.25 µg/ml).
6. Standard 2: Take 1 ml of 0.25 µg/ml and add 1 ml of diluent solution (equivalent to 0.125 µg/ml).
7. Standard 1: Take 1 ml of 0.125 µg/ml and add 1 ml of diluent solution (equivalent to 0.0625 µg/ml).
8. Inject 100 µl of each solution onto the HPLC system.

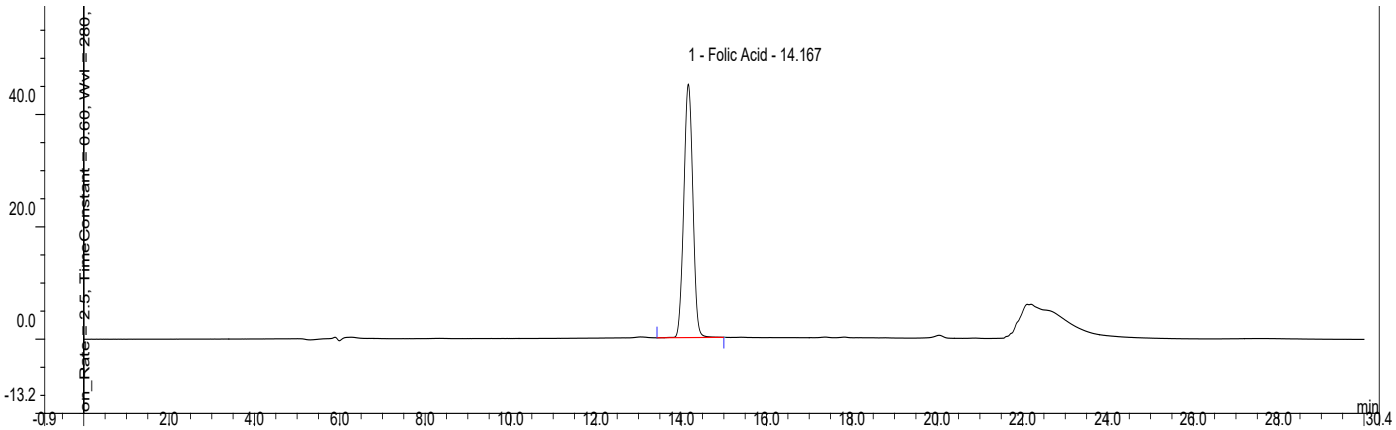
Recommended HPLC Conditions

HPLC Conditions			
Guard Cartridge	AQUASIL C18 3 µm, 4 mm x 10 mm or equivalent		
Analytical Column	AQUASIL C18 3 µm, 4.6 mm x 150 mm or equivalent		
Mobile Phase	Solution A: 0.1 % TFA in Water Solution B: Acetonitrile Prepare fresh on day of analysis.		
Gradient Conditions	Time (min)	% Solution A	% Solution B
	0	88	12
	2	88	12
	10	80	20
	15	80	20
	16	25	75
	20	88	12
	35	88	12
HPLC Pump	To deliver mobile phase		
Flow Rate	0.4 ml per minute		
UV Detector	280 nm		
Column Heater	Maintain guard and analytical column at 30 °C		
Integrator / Data Control System	From preferred supplier		
Injector	Autosampler / Rheodyne valve		
Injection Volume	100 µl		

Example HPLC Chromatogram for Infant Formula



Example HPLC Chromatogram for Cereal



Quality

RBR products are developed, manufactured, tested and dispatched under an ISO 9001 registered Quality Management System, guaranteeing a consistent product, which always meets our performance specifications. Our products have been used in many collaborative studies to develop standard European and International Methods and are widely used by key institutions, food companies and government laboratories. Customer references for RBR products are available on request.

Technical Support

RBR understand that from time to time users of our products may need assistance or advice. Therefore, we are pleased to offer the following services to our customers:

- Analysis of problem samples.
- Application notes for difficult samples.
- References from the RBR library.
- Installation and support of the KOBRA® CELL.
- Advice on detection parameters.
- Advice on preparation and handling of standards.
- Updates on legislation, sampling and other news by e-mail.
- Provision of spiked samples.

Please contact your local R-Biopharm distributor for further information.

Warranty

R-Biopharm Rhône Ltd makes no warranty of any kind, express or implied, except that all products made by R-Biopharm Rhône Ltd are made with materials of suitable quality. If any materials are defective, R-Biopharm Rhône Ltd will provide a replacement product. The user assumes all risk and liability resulting from the use of R-Biopharm Rhône Ltd products and procedures. R-Biopharm Rhône Ltd shall not be liable for any damages, including special or consequential damages, loss or expense arising directly or indirectly from the use of R-Biopharm Rhône Ltd products or procedures.

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