



February 2020

Biotin/Vitamin B7 in Oat and Grain Food ~ Manual and Automated ~

Do you have a special matrix that we should test for mycotoxins? Please let us know and write an e-mail to: info@LCTech.de

Sample Preparation

MYCOTOXINS

Oats and Grain Food

For many people a good day starts with a balanced breakfast. Besides coffee, bread rolls, sausage or scrambled eggs, cereals are also part of the morning meal for many people. More and more people are opting for the vitamin-rich alternative of oat or oatmeal instead of standard cereals. Whether as porridge, or as an ingredient in a smoothie - oats have become indispensable on many German breakfast tables.

Oat is a plant genus of sweet grasses and is mainly grown in regions with a moderate climate and high rainfall, for example in the low mountain ranges, in the foothills of the Alps or in coastal regions. Not only because of the local cultivation oat is considered as THE German superfood. The grain is also rich in proteins, fibres, minerals and vitamins, including biotin and vitamin B7.

SPE Clean-up Columns for the Analysis of Biotin/Vitamin B7

Biotin is a naturally occurring vitamin that belongs to the family of B vitamins (B7) and is one of the water-soluble vitamins. It can be found ubiquitous in many foods. The biotin concentration supports a vitamin-rich diet and is consequently found in many vitamin drinks, vitamin tablets and food supplements. Egg yolk, soybeans and liver are amongst the foods that are high in biotin content.

In order to determine reliably and quickly the compliance of the declared biotin amounts, LCTech offers the affinity column BioteX for the analysis of different matrices and foods. The BioteX affinity columns allow a high sample throughput and parallel sample preparation in food analysis via UV determination or in HPLC or LC/MS analysis. The columns are available in a convenient 3 mL polypropylene format and can be stored refrigerated for 9 months from the date of manufacture.

On the following page you will find a processing protocol with the use of a BioteX affinity column.

Processing Protocol

Homogenise 10 g oats/grain food and extract the mixture with 50 mL water. To ensure high extraction efficiencies, perform the extraction for at least 10 – 30 minutes. The extraction can also be carried out in phosphate buffer.

Filter the raw extract and dilute 2.5 mL with 47.5 mL PBS. Load the sample onto the Affinity Column BioteX and wash the column with 10 mL deionized water.

Elute the biotin with 2 mL methanol. Make sure that the column bed is incubated with Methanol for 5 minutes in order to ensure a fully release of Biotin from the column.

Finally, depending on the chromatographic conditions, the eluate can be concentrated, redissolved or injected.

Recovery Rates

Content of Biotin/Vitamin B7 in Oat/Grain food

Biotin/Vitamin B7	
Standard*	100
Recovery Rate** Oatmeal 2 ppm	98
Recovery Rate** Wheat flour 2 ppm	93

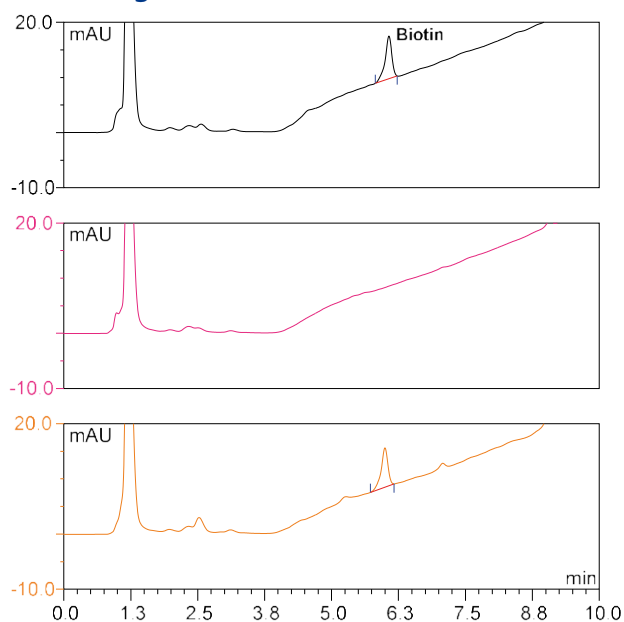
*Standard is set = 100 %, **Corrected with non-spiked sample

HPLC-Conditions

(Biotin/Vitamin B7)

Biotin/Vitamin B7	
HPLC:	gradient
Column Oven:	35 °C
Separation Column:	EC100/3 Nucleodur Phenyl-Hexyl, 3 µm
Flow Rate:	0.6 mL/min
Eluent:	0.1 % Phosphoric acid in HPLC - water (eluent 1) 0.1 % Phosphoric acid in Acetonitrile (Eluent 2)
Gradient:	0 - 2 minutes Eluent 1, 100 % 2 - 12 minutes Eluent 1, 90 %, Eluent 2, 10 % Gradient graph 5 12 -15 minutes Eluent 1, 100 %
UV - Detection	215 nm
Injection volumes	75 µL

Chromatograms



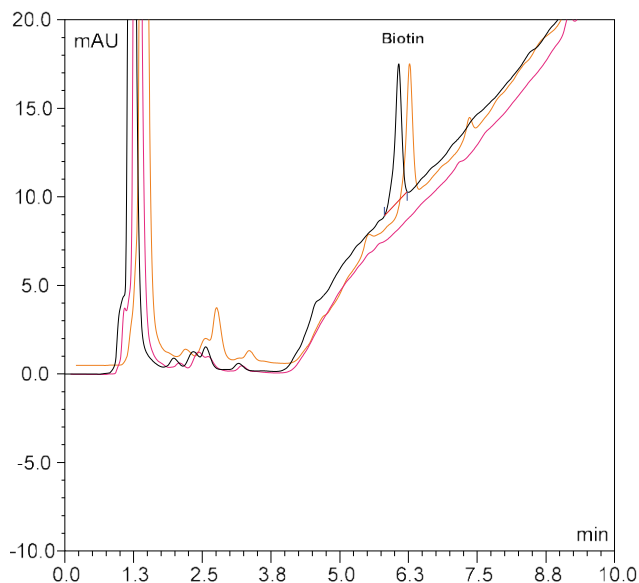
Black: 1 µg/2 mL Biotin, concentrated, in 400 µL of solvent redeemed

Red: Oatmeal not spiked, eluate constricted, in 400 µL of solvent redeemed

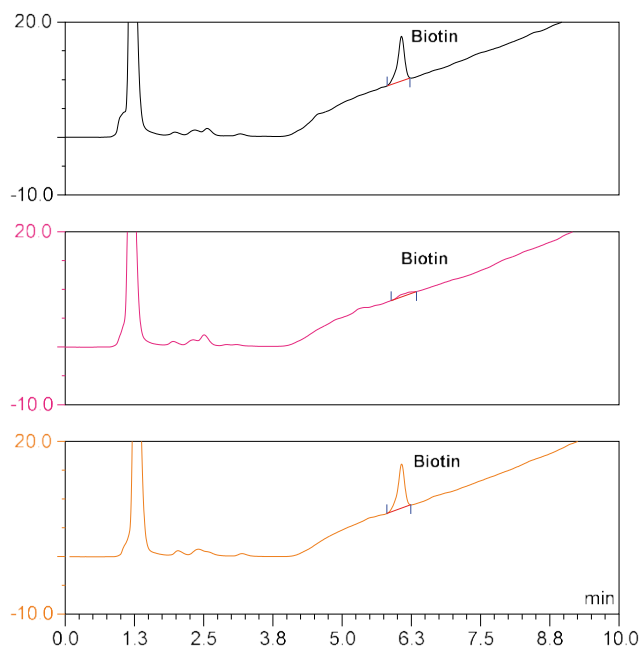
Orange: Oatmeal spiked with 2 ppm biotin, eluate constricted, in 400 µL of solvent redeemed



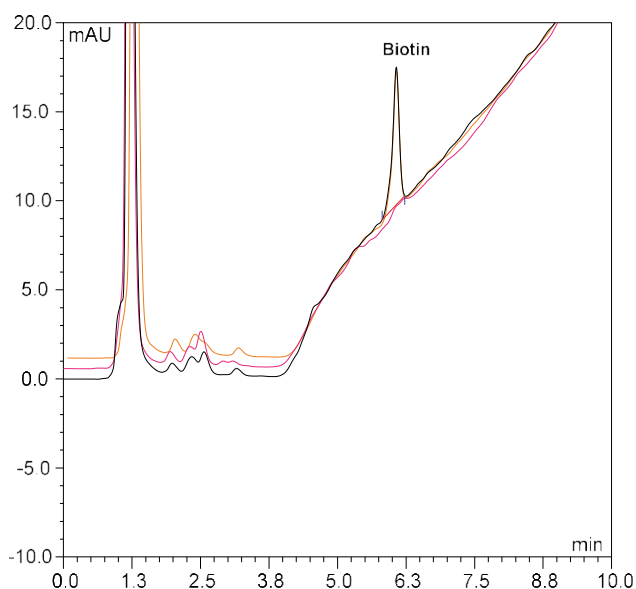
Affinity column BioteX for clean-up of biotin/vitamin B7



Black: 1 μg concentrated, in 400 mL of solvent redeemed
Red: Oatmeal not spiked, eluate constricted, in 400 μL of solvent redeemed
Orange: Oatmeal spiked with 2 ppm biotin, eluate constricted, in 400 μL of solvent redeemed



Black: 1 $\mu\text{g}/2\text{ mL}$ Biotin, constricted, in 400 mL of solvent redeemed
Red: Wheat flour, not spiked, eluate constricted, in 400 μL of solvent redeemed
Orange: Wheat flour, eluate constricted, in 400 μL of solvent redeemed



Black: 1 $\mu\text{g}/2\text{ mL}$ Biotin, constricted, in 400 mL of solvent redeemed
Red: Wheat flour, not spiked, eluate constricted, in 400 μL of solvent redeemed
Orange: Wheat flour, eluate constricted, in 400 μL of solvent redeemed



Automated Sample Preparation with FREESTYLE SPE

The entire clean-up process described above can be performed fast and easy by using the FREESTYLE SPE. Position the oat sample in the FREESTYLE SPE. Change settings or parameters in the software with a few mouse clicks and start the system.

These LCTech Products were used:

Affinity column BioteX
P/N 14100 / 14101

FREESTYLE SPE Robotic System for Automated
Sample Preparation
P/N 12663/12668