

Matrix of the Month

March 2015:

**Biotin in vitamin tablets
via the affinity column
BioteX
- 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!

About Biotin

Biotin is a naturally occurring vitamin which belongs to the family of the B vitamins (B7). Furthermore it belongs to the water soluble vitamins and is found ubiquitously in many foodstuff. The concentration of Biotin (according to LMIV EU Nr. 1169/2011) supports a vitamin rich diet and is found in various vitamin drinks, tablets or other food supplementation. The affinity column BioteX enables an effective clean-up with different sample material and has been developed for the parallel sample preparation within the food analysis via UV-detection for analysis with HPLC or LC/MS.

Automated Sample Preparation with FREESTYLE SPE

**Especially fast ...
Especially simple ...**



With FREESTYLE SPE you process your sample fully automated around the clock, even on weekends. So your sample is always in time prepared for the particular analysis.

Any manual method that has proved successfully in your laboratory, can be automated without any problems. With FREESTYLE SPE you can process many different SPE-column formats from 1 to 15 mL. And therefore the 3 mL BioteX affinity column of LCTech, too.

The application parameters are identical to the manual processing, but is done autonom and fully automated. These and other advantages provide FREESTYLE SPE:

- No cross-contamination
- Extremely fast and precise processing
- Excellent recovery rates
- Very simple and intuitive software



**The FREESTYLE SPE
with the EVAporation Module**

Manual Processing →

Protocol of Manual Processing

Extract 1 g of a thoroughly homogenized vitamin tablets with 100 mL of the extraction solution (PBS buffer) in a blender jar at high speed, e.g. with an Ultraturrax.

Pass the extract through a plaited filter. Dilute 25 mL of the filtered extract with 25 mL PBS-Puffer (pH 7.2). If there is any precipitation during mixing with the buffer, practically the sample volume has to be filtered by means of a glass fiber filter or centrifuged. After opening the column, drain the storage buffer until the level reaches the upper frit.

Take 10-50 mL of the diluted extract (depending on the sensitivity of the detection) and pass it through the affinity column Biotex. A maximum load of 1000 ng Biotin should not be exceeded. A gentle vacuum or overpressure may be used in all steps passing liquid through the column; nevertheless, it is indispensable to maintain a maximum flow rate of 2 mL/min. Let all of the sample drain through the column until there is no more sample in the column, a complete drying of the column must be avoided at this stage! Wash the column with 10 mL of distilled water. Residual water is now removed by a gentle gas stream or vacuum.

Elute with 1 mL of methanol; let the methanol act on the gel for 5 minutes to break the analyte-ligand bond. Collect the eluate in a 2 mL measuring cylinder and adjust the volume to 1 mL.

Dilute or concentrate the eluate to your requirements and measure directly by HPLC.

HPLC Conditions

Biotin

HPLC: Gradient

Column oven: 35 °C

Separation column: EC100/3 Nucleodur Phenyl-Hexyl, 3 μ L

Flow rate: 0.6 mL/min

Injection volume: 100 - 100 μ L

UV-Detector: 215 nm

Eluent 1: 1% Phosphoric acid = HPLC-Water

Eluent 2: 1% Phosphoric acid = Acetonitrile



Affinity column Biotex

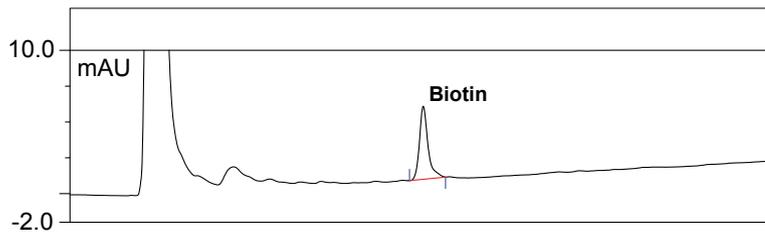
Recovery Rates

Content of Biotin in vitamin tablets	
Biotin	
Standard*	100
Recovery rate** vitamin tablets 10000 μ g Biotin	90

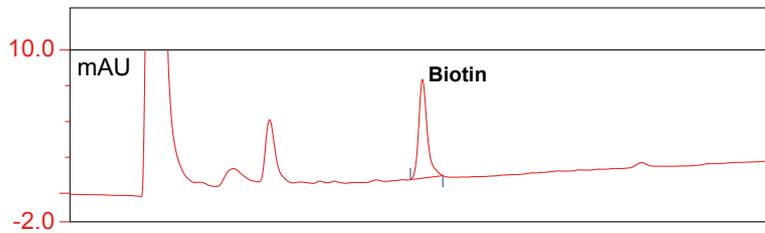
* Standard is set = 100 % , ** corrected with non-spiked sample

Chromatograms →

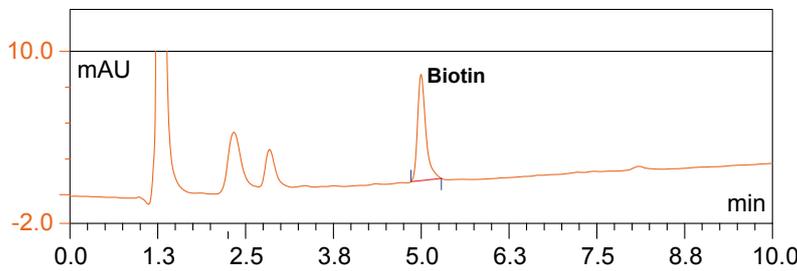
Chromatograms



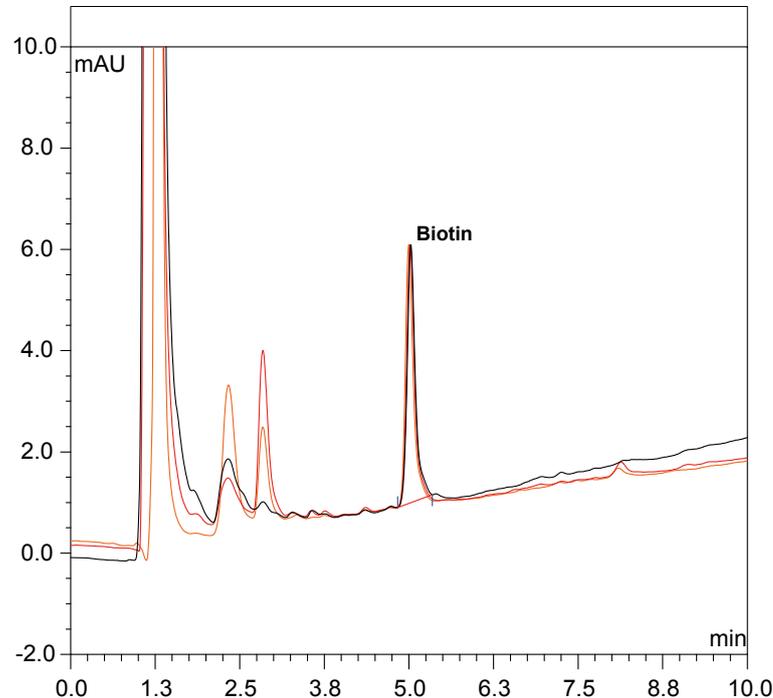
0.5 µg BioteX cleaned-up via FREESTYLE SPE (black).



0.5 µg Biotin cleaned-up via BioteX manual (red).



Standard 0.5 µg Biotin (orange).



Overlay of chromatograms.
Detection via UV with 215 nm.



These LCTech products were used:

BioteX affinity column,
for Biotin / Vitamin B7 analysis

P/N 14100 / 14101

FREESTYLE SPE
Robotic system for
sample preparation

P/N 12663, 12668

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Do you have further questions?
Please just write an e-mail to info@LCTech.de