

## Determination of Orotic acid in milk

**Method** VFD0134J\_E  
**HPLC**

**Column:** Eurokat H, 10 µm, 300 x 8 mm

**Order No.** 30GX340EKN

**Phase:** Eurokat H, 10 µm

**Eluent:** 0.01 N Sulfuric acid  
**Gradient:** Isocratic  
**Flow rate:** 0.6 ml/min  
**Temperature:** 60 °C  
**Volume:** 20 µL (Standard)

**Detection:** UV, 210 nm

**Substances:** Orotic acid, Lactic acid and Acetic acid

**Keywords:** Orotic acid, Eurokat H, Vitamin B13, dietary supplement

**Sample preparation:** Five gram of milk were filled up to 25 ml with 4 mM H<sub>2</sub>SO<sub>4</sub>, centrifuged for 10 min with 2000 rpm, filtrated (0,45 µm) and diluted 1:10 before 5 µl were injected for analysis.

### Chromatogram:

**Milk sample:**

1 Impurity  
2 Orotic acid  
2,2 µg/ml

**Standard:**

1 Orotic acid  
15,6 µg/ml  
2 Lactic acid  
3 Acetic acid

