Lovastatin and citrinin in red yeast rice products

Dr. Ines Klingelhöfer and Prof. Dr. Gertrud Morlock

The Food Science group at Justus Liebig University Giessen, Germany, develops straightforward HPTLC methods as the following one.

Introduction

Lovastatin (Mevinolin, Monakolin K) is synthesized by *Monascus* fungi strains during fermentation of rice. At that point the carcinogenic, mutagenic and antibiotic mycotoxin citrinin can be formed. Lovastatin acts as lipid-lowering agent and may be present in the water-soluble hydroxy acid (LH) and slightly fat-soluble dehydrolyzed lactone form (LL).

A newly developed HPTLC method was validated and applied to 19 red yeast rice products, including powders, food supplements, and Chinese proprietary medicines (Xuezhikang and Zhibituo). The simultaneous analysis including sample preparation allows a high throughput of matrix-rich samples (10 min and 0.5 Euro per analysis) [1].

Chromatogram layer

HPTLC plate silica gel 60 (Merck), 20 × 10 cm

Standard solutions

LL (100 µg/mL) and citrinin solutions (10 µg/mL) in acetone; alkaline hydrolysis of LL (200 µL LL of 1 mg/mL, 700 µL acetone and 50 µL 1 N sodium hydroxide) for 30 min at 4 °C; 50 µL 1 N acetic acid added for neutralization (200 µg/mL LH)

Sample preparation

Samples (30 mg) extracted in 1 mL (if completely water-soluble) or 2 mL water – acetone 2:3 (*V*/*V*), vortexed (30 s) and centrifuged (9600*xg*, 5 min)

Sample application

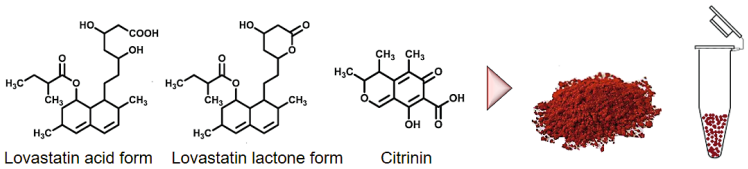
Bandwise with the Automatic TLC Sampler (ATS4), band length 6 mm, track distance 8 mm, application volume 0.5–10 µL/band (standards) and 0.5–20 µL/band (samples)

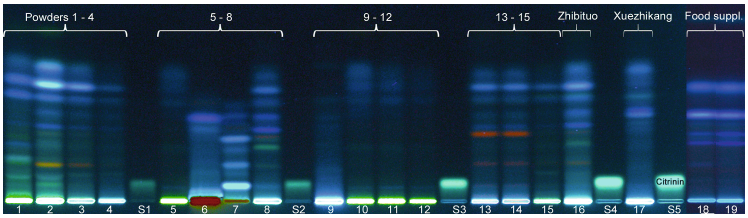
Chromatography

In Twin Trough Chamber 20 × 10 cm, using *n*-hexane – acetone – 10% acetic acid, 6:4:0.1, up to 65 mm

Densitometry

TLC Scanner 4 with winCATS, multi-wavelength scan of absorbance of LL/LH at 238 nm and fluorescence of citrinin at 313/>400 nm.





HPTLC chromatogram of red yeast rice products at UV 366 nm (lovastatin not visible)

Results and discussion

Method validation was successfull. For three analytes at three concentration levels including sample preparation, the overall mean recovery rate was 109.9% ± 5.9% and the mean intermediate precisions were ≤ 2.6% in red yeast rice matrix. For lovastatin analysis, the method was applied to 15 powders (1.5–26.2 g/kg), Zhibituo tablet (2.7 g/kg), Xuezhikang capsule (11.1 g/kg) and two food supplements (40.7 and 41.4 g/kg), which took 10 min and 0.5 Euro per sample [1].

[1] I. Klingelhöfer, G. Morlock, Anal Bioanal Chem 25 (2019) 6655

Further information is available on request from the authors.

Contact: Prof. Dr. Gertrud Morlock and Dr. Ines Klingelhöfer, Justus Liebig University Giessen, Heinrich-Buff-Ring 26–32, 35392 Giessen, Germany, Gertrud.Morlock@uni-giessen.de