



Patulin in juice and solid samples by HPLC

Scope: This is an HPLC method for the determination of patulin in fruit juices (apple, pear, cherry, etc.) and solid samples (apple puree, etc.)

1. Extraction

A. Liquid samples

1. Pipet 4.0 mL of juice sample into a 50 mL screw-capped tube. Add 21.0 mL acetonitrile.
2. Close tube and mix with a vortex mixer at high speed for 60 seconds.

B. Solid samples

1. Weigh 25 g sample into a 250 mL screw-capped Erlenmeyer flask.
2. Add 100 mL of acetonitrile:water (84+16) and close the Erlenmeyer.
3. Shake for 1 hour on gyratory shaker.
4. Filter the extract through high speed qualitative paper into a container.

2. Purification

A. Liquid samples

1. Pour about 10 mL of sample extract on the test tube provided with the Micotox® M2008 patulin column.
2. Slowly push the Micotox® M2008 cleanup column (rubber flange end) into the test tube creating a tight seal between rubber flange and glass wall of tube. Push until just a little bit more of 5 mL purified extract are filtered into the column reservoir. Avoid passing an excessive volume of extract through the purification column because it may become saturated and interferences may elute.
3. Transfer exactly 5.0 mL of purified extract into a silanized vial (e.g. Pierce® Reacti-Vials with conical bottom) or silanized test tube.
4. Evaporate to dryness under a gentle stream of nitrogen or using vacuum and heat (max. 60°C). Remove vial just as evaporation is nearing completion in order to avoid patulin degradation.
5. Dissolve residue with 400 µL of acetonitrile:water at pH 4 (1+9). Adjust water pH with acetic acid. Sonicate or mix with vortex for 30 seconds. NOTE: Patulin is unstable as a dry film. Immediately dissolve the residue in mobile phase to avoid low recovery rates. Store test solution in freezer if LC analysis is delayed.
6. Filtrate the dissolved extract through a 0.45 µm pore filter and inject 100 µL into HPLC.



B. Solid samples

1. Pour about 10 mL of filtered sample extract on the test tube provided with the Micotox® M2008 patulin column.
2. Slowly push the Micotox® M2008 cleanup column (rubber flange end) into the test tube creating a tight seal between rubber flange and glass wall of tube. Push until just a little bit more of 4 mL purified extract are filtered into the column reservoir. Avoid passing an excessive volume of extract through the purification column because it may become saturated and interferences may elute.
3. Transfer exactly 4.0 mL of purified extract into a silanized vial (e.g. Pierce® Reacti-Vials with conical bottom) or silanized test tube.
4. Evaporate to dryness under a gentle stream of nitrogen or using vacuum and heat (max. 60°C). Remove vial just as evaporation is nearing completion in order to avoid patulin degradation.
5. Dissolve residue in 400 µL of acetonitrile:water at pH 4 (1+9). Adjust water pH with acetic acid. Sonicate or mix with vortex for 30 seconds. NOTE: Patulin is unstable as a dry film. Immediately dissolve the residue in mobile phase to avoid low recovery rates. Store test solution in freezer if LC analysis is delayed.
6. Filtrate the dissolved extract through a 0.45 µm pore filter and inject 100 µL into HPLC.

3. HPLC

Standard solution:

1. Patulin stock solution: 25 µg/mL in acetonitrile.
2. Working standard: Prepare a diluted standard by adding 20 µL of the 25 µg/mL patulin standard solution (0.5 µg patulin) to 5.0 mL of mobile phase (0.8% THF in water). This solution corresponds to 100 ng/mL. Transfer 1 mL of working standard into an amber autosampler vial for standard injections.
3. Inject 100 µL into HPLC (10 ng on column), equivalent to 50 ng/mL patulin in liquid samples, and 40 ng/g in solid samples).

Column: Spherisorb ODS (3.9 mm x 25 cm, 5 µm C-18).
NOTE: It is recommended to evaluate the LC column performance by injecting 50-100 µL of a solution containing both patulin and 5-hydroxymethylfurfural (HMF), e.g. 1.0 µg/mL each. The patulin and HMF should elute as two separate peaks. If the HMF and patulin are not completely separated use a different kind of column. Analysis cannot be performed unless these two peaks are well separated.

Mobile phase: 0.8% tetrahydrofuran (THF) in water.
Flow rate: 1 mL/min.
Detector: UV at 276 nm (245 nm can also be used).



Retention time: 10.0 minutes (approximately). When 5-hydroxymethylfurfural is also injected, its retention time is 8.0 minutes approximately.

Calculations

Liquid samples:

$$W = 4 \text{ mL} \times (5 \text{ mL} / 25 \text{ mL}) \times (0.1 \text{ mL} / 0.4 \text{ mL}) = 0.2 \text{ mL}$$

Amount of patuline standard injected on column: 10 ng, equivalent to 50 ng/mL (50 µg/L) on sample (10 ng/0.2 mL = 50 ng/mL).

Solid samples:

$$W = 25 \text{ g} \times (4 \text{ mL} / 100 \text{ mL}) \times (0.1 \text{ mL} / 0.4 \text{ mL}) = 0.25 \text{ g}$$

Amount of patuline standard injected on column: 10 ng, equivalent to 40 ng/g (40 µg/kg) on sample (10 ng/0.25 g = 40 ng/g).