Quantitation of glycerol in tobacco by HPTLC

A-41.3

Key words

- Instrumental HPTLC
- Quantitative analysis
- Densitometry (fluorescence)
- Food chemistry
- Humectant
- Glycerol

Scope

Tobacco leafs are treated with glycerol to maintain stability and the desired pliability for further processing. Regulations require that the content of humidifying agents is monitored during storage.

Glycerol is extracted from tobacco with acetone - water. The extract is chromatographed on silica gel with acetone - water 98:2 and post chromatographic derivatized with lead acetate and 2',7'-dichlorofluoresceine. After stabilizing the fluorescence intensity the plate is evaluated by densitometry.

Required or recomended CAMAG devices

Automatic TLC sampler 4 or Linomat 5

Twin Trough Chamber 20 x10 cm or 10x10 cm

Chromatogram Immersion Device

DigiStore

TLC Scanner 3 and winCATS software

Chemicals

Acetone, glycerol, lead (IV) acetate, glacial acetic acid, 2‘,7‘-dichlorofluoresceine,ethanol, toluene, parafiin oil, hexane,methanol

HPTLC plates silica gel 60 F 254 (Merck), 20x10 or 10x10 cm. Plates are pre-washed by development with methanol to upper edge, then dried at 120°C for 30 min.

Sample

About 2 g sample are dried for 30 min at 100°C in an oven. 0.5 g of the dry sample are shaken with 6 mL water and 20 mL acetone for 5 min. 0.1 g activated carbon is added and the mixture is shaken again for 5 min. The mixture is centrifuged at 3000 rpm for 5 min. If the solution is still not clear, it can be filtered through filter paper.

Standard

60 mg glycerol are dissolved in 100 mL of an acetone-water mixture (98:2). 1 µL of the solution contains 0.6 µg glycerol.

Sample application

1 µL of samples and 1, 3, 5, and 7 µL of the standard solution are applied with the CAMAG Automatic TLC Sampler 4 or Linomat 5 as 6 mm bands, 8 mm from the lower edge of the plate, and at least 10 mm from the sides.

Chromatography

Mobile phase: Acetone, water (98:2)

Development: 10/5 mL developing solvent are placed in each trough of a 20x10/10x10 cm Twin Trough Chamber, containing a filter paper on one side. The lid is closed and the chamber allowed to saturate for 30 min. Developing distance is 6 cm measured from lower edge of plate. The plate is dried in a stream of cold air.

Derivatization reagents

Lead-dichlorofluoresceine solution (must be prepared fresh for each plate) (LDS)

Solution A: 2 g lead (IV) acetate are dissolved in 10 mL glacial acetic acid.
Solution B: 1 g 2‘,7‘-dichlorofluoresceine is dissolved in100 mL ethanol
5 mL each of solution A and B are added to 190 mL toluene.

Paraffin oil-hexane solution (PHS): 40 mL paraffin oil are dissolved in 200 mL hexane.

Detection

Using the Chromatogram Immersion Device the plate is dipped in LDS for 6s, dried in a stream of cold air for 1 min, then dipped in PHS for 1s and dried a stream of cold air.

Documentation

With CAMAG DigiStore under UV 366 nm.

Densitometry

With CAMAG TLC Scanner 3 and winCATS software, fluorescence measurement at 366/>400 nm (Hg lamp).

Results screening

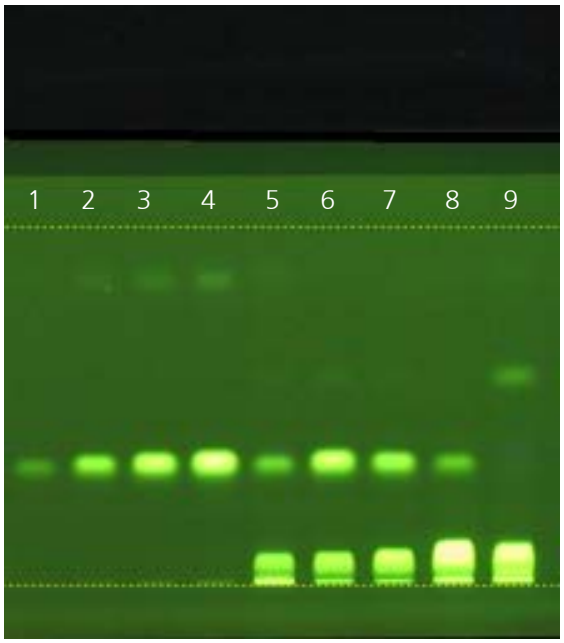
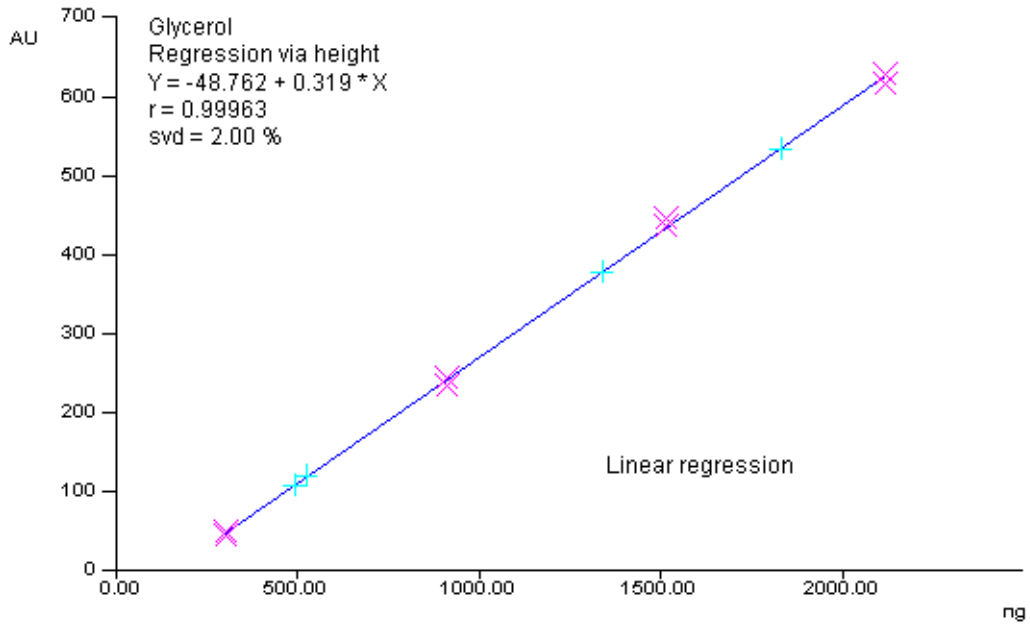


Image after derivatization under UV 366 nm
1-4: Glycerol standard, increasing volume
5: Marlboro light (Switzerland)
6-8: Aromatized water pipe tobacco (Egypt)
9: Pipe tobacco (Virginia, USA), contains no glycerol



Calibration for glycerol
Red: Standard levels (duplicates)
Blue: samples

Note: The presented results are to be regarded as examples only!