

# HPLC Determination of Free Amino Acids and Protein Amino acids in Tobacco

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## A. FREE AMINO ACIDS

1. For determination of free amino acids, weigh 0.5 g tobacco sample into a 60 ml polyethylene bottle and add 20 ml 0.1N HCl solution.
2. Shake for 20 minutes on a high speed shaker.
3. Filter a small amount of the extract solution through a 0.45  $\mu$ m filter.
4. Derivatize 40  $\mu$ l of the filtrate with 140  $\mu$ l derivatizing reagent (ethanol:triethylamine:water:phenylisothiocyanate = 110:20:8:2) in a sample vial.
5. Agitate the sample with a vortex shaker and stand for 30 minutes at room temperature.
6. Dry the sample in a Savant speed vac concentrator with heating on.
7. Dissolve the sample with 1 ml acetonitrile:water (7:2).
8. Derivatize the standard in the same manner as the sample (Standard solution: Asp 0.4 mg/ml, Glu 0.2 mg/ml, Asn 0.5 mg/ml, Gln 0.2 mg/ml, and Pro 0.4 mg/ml)
9. Inject 5  $\mu$ l samples and 3  $\mu$ l or 5  $\mu$ l standard.

**Column:** Waters Pico.Tag column for amino acid analysis (Part No. 88131).

**HPLC:** Perkin-Elmer Series 4 LC with an UV Detector.

### HPLC running conditions:

Eluent A: 13.6 g sodium acetate + 0.8 ml triethylamine/liter (pH = 6.4).

Eluent B: acetonitrile:water (6:4).

Flow rate: 1 ml/minute.

Gradient: 4-49% B (0-10 min, curve 0.2), 49%-100% B (10-10.5 min, linear), 100% B (10.5-14.5 min.), 100%-4% B (14.5-15 min), 4% B (15-19 min).

**Detection:** UV at 250 nm.

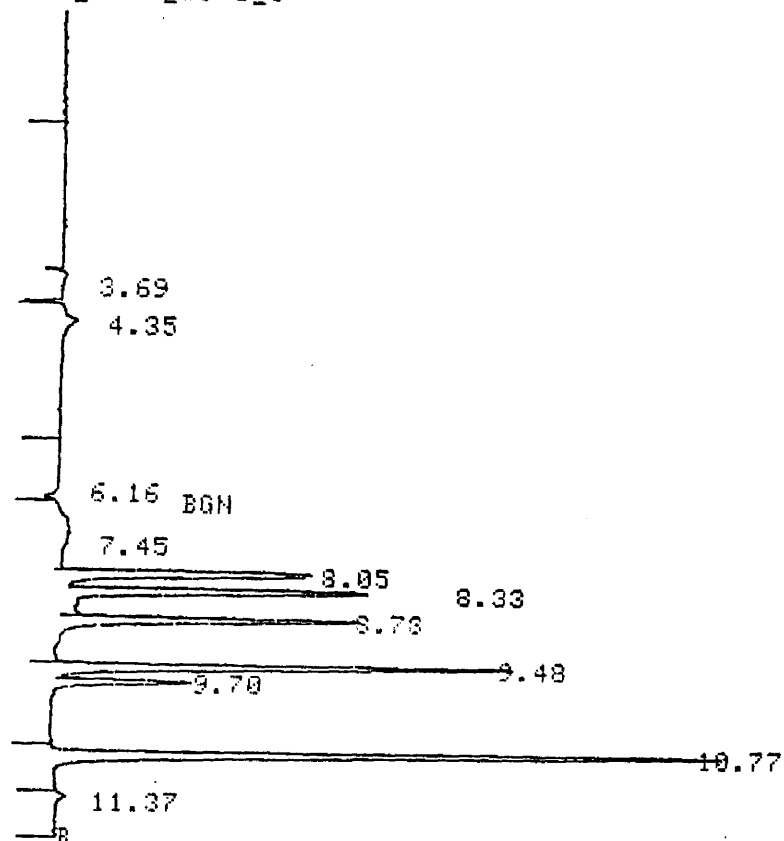
## B. PROTEIN AMINO ACIDS

1. For determination of protein amino acids, weigh 100 mg tobacco sample into a protein hydrolysis tube.
2. Add 6 ml 6N HCl, replace the air with nitrogen gas and heat the tube on a dry bath for 23 hour at 110°C.
3. Dilute the sample with 4 ml distilled water.
4. Filter the sample through a 0.45  $\mu$ m filter.
5. Derivatize 100  $\mu$ l filtrate with 140  $\mu$ l derivatizing reagent shown above.

6. Derivatize amino acid standard (Pierce amino acid standard H, No. 20088) with 140  $\mu$ l derivatizing reagent shown above.
7. Dissolve the sample and standard with 1 ml acetonitrile:water (7:2).
8. Inject 5  $\mu$ l aliquot into a HPLC.

All equipment and analytical conditions are the similar to those for determination of free amino acids except that a YMC-Pack ODS-A column (250 x 4.6 mm I.D., Cat. No. A-303-5) is used.

W-6 A-16 C-10 O-5



FILE 75 RUN 1 STARTED 00:21.1 80/02/17 FREE  
XS METHOD 1 FREE AMINO ACIDS LAST EDITED 00:00

