Determination of Total Desulfoglucosinolates in Canola Seed by Ultra High Performance Liquid Chromatography with Ultraviolet Detection.

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Abstract
The objective was to validate an analytical method for the determination of total glucosinolates using UHPLC-UV detection.

Test Matrix
The method was validated using a commercially available canola seed sample. Canola samples were ground with dry ice, placed in a 15 mL disposable glass culture tube, and weighed into a 25mL volumetric flask and filled to volume with deionized water.

Chemicals and Reagents
Sodium Acetate, Fisher, Fair Lawn, NJ
Imidazole, Sigma Aldrich, St. Louis, MO
Methanol (MeOH), HPLC Grade, EMD
Formic Acid (FA), Fisher, Fair Lawn, NJ
Acetonitrile (ACN), HPLC Grade, EMD, Billerica, MA
Acetic acid, HPLC Grade, Millipore, MA
AnalR Acetone, VWR
Helix pomatia

Preparation of desulfation columns:
1. Place a column of ion exchange resin in a column holder and rinse with 50 mL of deionized water.
2. Rinse the column with 50 mL of 1% (v/v) acetic acid.
3. Rinse the column with 50 mL of ACN.
4. Rinse the column with 100 mL of 1 M sodium acetate buffer.
5. Rinse the column with 50 mL of 1% (v/v) acetic acid.
6. Rinse the column with 50 mL of 1% (v/v) formic acid.
7. Rinse the column with 50 mL of ACN.
8. Rinse the column with 50 mL of deionized water.

HPLC and UHPLC Mobile Phase Gradient Program
Both the UHPLC and HPLC-UV detection mobile phase gradient programs were based on a linear gradient of MeOH and water.

UHPLC Mobile Phase Gradient Program
- MeOH (A) and H2O+0.1% Formic Acid (B)
- %A ranges from 5% to 100%
- Gradient time: 13 minutes
- Flow rate: 0.200 mL/min
- Column: Waters Acquity UPLC BEH Shield RP18, 2.1 x 100 mm, 1.7 µm
- Injection Volume: 40 µL
- Wavelength: 229 nm
- Temp: 20°C
- Pressure: 2000 bar
- Sample size: 500 µL

UHPLC Mobile Phase Gradient Program
- MeOH (A) and H2O+0.1% Formic Acid (B)
- %A ranges from 0% to 100%
- Gradient time: 30 minutes
- Flow rate: 1.0 mL/min
- Column: Waters Acquity UPLC BEH Shield RP18, 2.1 x 100 mm, 1.7 µm
- Injection Volume: 20 µL
- Wavelength: 229 nm
- Temp: 20°C
- Pressure: 3000 bar
- Sample size: 500 µL

References