Sterol analysis

1. Freeze-dried plant tissues (10 mg) were extracted three times with 2 ml of $CHCl_3$-MeOH (1 : 1).
2. 2 μg of [25,26,26,26,27,27,27-$^2H_7$] Cholesterol (Cambridge Isotope Laboratories, Inc. (www.isotope.com)) used as an internal standard was added directly to the sample homogenate.
3. The extract was dried and chromatographed on a silica gel cartridge column (Sep-Pak® Vac 500 mg/6 cc, Waters) with 8 ml of hexane-EtOAc (2 : 1) and 8 ml of $CHCl_3$-MeOH (1 : 1).
4. The hexane-EtOAc eluent, in which fatty acid ester and free sterols were included, was dried and saponified with 1 ml each of MeOH and 20% KOH aq. for 1 h at 80 °C.
5. The $CHCl_3$-MeOH eluent, in which sterol glucosides were included, was dried in a rotary evaporator.
6. The residue and extraction debris, in which inclusion sterols in polymer like starch were included, were combined and hydrolyzed with 1 ml each of MeOH and 4 N HCl for 1 h at 80 °C.
7. These reaction mixtures were then extracted three times with 2 ml of hexane, and the combined hexane layer was evaporated to dryness.
8. The residue was trimethylsilylated with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (/400 μl, Aldrich) at 80 °C for 30 min and analyzed using GC-MS.
9. GC-MS analysis was carried out under the following conditions: a mass spectrometer (JMS-AM SUN200, JEOL) connected to a gas chromatograph (6890A, Agilent Technologies), EI (70 eV), source temperature 250 °C, DB-1 column (15 m × 0.25 mm, 0.25-μM film thickness, J&W Scientific), injection temperature 250 °C, column temperature program: 80 °C for 1 min, then raised to 280 °C at a rate of 20 °C min^{-1}, and held at this temperature for 8 min; interface temperature 300 °C, carrier gas He, flow rate 1 ml min^{-1}, splitless injection. The endogenous levels of sterols were determined as the peak area ratios of molecular ions for the endogenous one and for the internal standard.

TIPs:

Be careful to contamination of cholesterol from your hands.
Don't touch your samples and glass wares directly!
Don't use latex glove to avoid phytosterols contamination!
We usually use NeoPro, MICROFLEX (#NPG-888-M). This globe is non-latex.