

GC Application

ID No.: 16528

Sterols pH from Olive Oil on ZB-5MS

Column: Zebtron[™] ZB-5MS, GC Cap. Column 30 m x 0.25 mm x 0.25 µm, Ea

Phase: 5% Phenyl-Arylene 95% Dimethylpolysiloxane

Dimensions: 30 meters x 0.25 mm x 0.25 µm

Order No: 7HG-G010-11

Oven Profile: From 220 °C to 330 °C at a ramp of 25 °C/min and hold for 5 min.

Carrier Gas: Constant Flow Helium, 1.5 mL/min

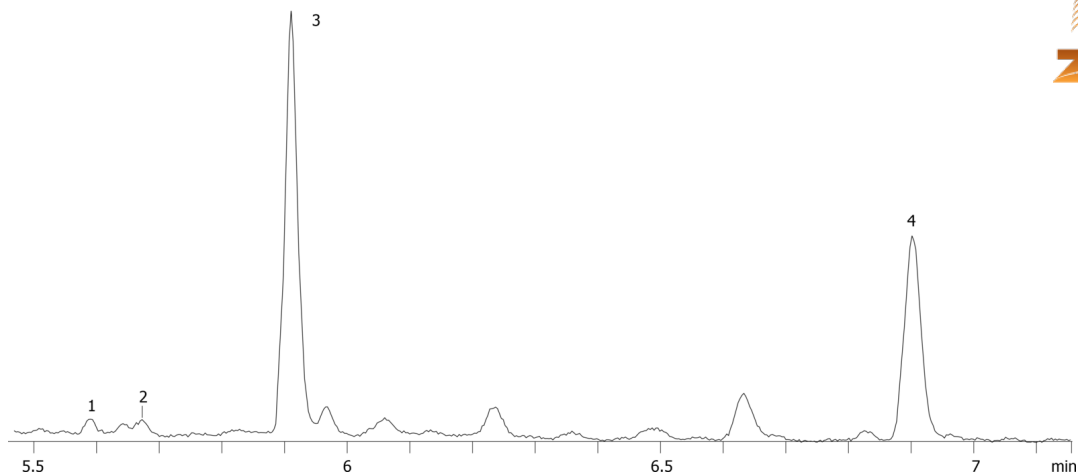
Injection: Splitless :1 0.5 µL @ 275°C

Detection: Mass Spectrometer (MS) (275°C)

16528



Products used in this application:



ANALYTES:

- 1 Campesterol
- 2 Stigmasterol
- 3 b-Sitosterol
- 4 Betulin (internal standard)



Sample Preparation Details

for GC Application ID No.: 16528

Sterols pH from Olive Oil on ZB-5MS

PRODUCT DESCRIPTION:

Strata® C18-E (55 µm, 70 Å), 1 g / 6 mL, Tubes , 30/Pk

Order No.: 8B-S001-JCH

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a 1 g bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

Shake and reflux 0.7 g of olive oil in 10 ml 0.5 M KOH in ethanol at 77 °C for 20 min. At room temperature add to sample prep 10 ml 0.22 mg/ml betulin (istd) in chloroform and shake for 10 min. In a 20 ml sample vial pH (2-) 3 ml liquid phase to 2.4 with 5 M HCl in ethanol dropwise, then transfer and filter to a BD 10 ml needles syringe screw to a Phenex 30 mm 0.45 µm teflon filter and collect filtrate in a 4ml screw cap sample vial. Set up and condition a Strata C18-E (55 µm, 70 Å) sorbent onto a manifold, plugged to a vacuum pump, and sorbent cartridge pointing into a 1/125 mm culture tube, with 5 ml methanol and 5 ml chloroform. Replaced culture tube with a new and label accordingly, then transfer 1 ml filtrate onto miniscuos chloroform-sorbent and elute sterols with 15 ml 5 % methanol in chloroform. Concentrate sterols to 1 ml by blowing nitrogen onto eluant, then transfer 200 ul into a 500 ul insert sample, dry with nitrogen, reconstitute with 100 ul pyridine, and derivatize with 100 ul BSTFA:TMCS; 99:1. Warm up to 70 °C for 20 min and inject.

Wash:

Dry:

Elute:

Final Prep and Analysis:

Initial Sample Weight / Unit: 0.70 grams

Inject: 0.5 µL on HPLC Mass Spectrometer (MS) @ 250-500 amu (275°C)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Campesterol	0				
2 Stigmasterol	0				
3 b-Sitosterol	0				
4 Betulin	0				

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals. Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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For more information contact your Phenomenex Representative at info@phenomenex.com



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