

## Sterols from Olive Oil by FID on ZB-5HT

**Column:** Zebtron™ ZB-5HT, GC Cap. Column 30 m x 0.25 mm x 0.10 µm, Ea

**Phase:** 5% Phenyl 95% Dimethylpolysiloxane

**Dimensions:** 30 meters x 0.25 mm x 0.1 µm

**Order No:** 7HG-G015-02

**Oven Profile:** 220 °C to 350 °C @ 15 °C/min.

**Carrier Gas:** Constant Flow Helium, 2 mL/min

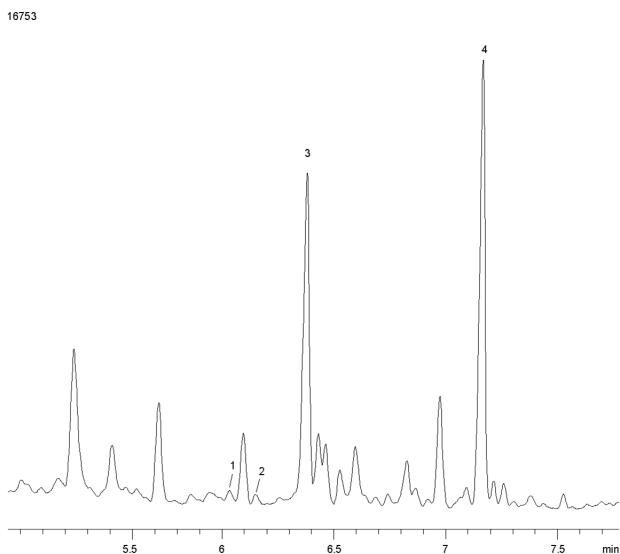
**Injection:** Splitless :1 0.5 µL @ 350°C

**Detection:** Flame Ionization (FID) (350°C)

**Analyst Note:** Sample Prep: Saponification, Solid Phase Extraction (SPE), and Derivatization.  
Recommended Accessories:  
Recommended Liner: Zebtron PLUS Single Taper Z-Liner, 4 mm ID  
Liner Part No.: AG2-0A13-05 (for Agilent systems)



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.: 16753

## Sterols from Olive Oil by FID on ZB-5HT

### 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:

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### Load:

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Saponify sample in 10 mL 0.5 M KOH in ethanol at 77 °C for 20 min.

When cool, add 10 mL of 0.22 mg/ml betulin (istd) in chloroform and shake.

Adjust 3 mL aliquot of sample to pH 2.5 with 5 M HCl.

Syringe filter aliquot through Phenex 30 mm 0.45 µm teflon filter.

Purify via Strata C18-E (55 µm, 70 Å).

[Condition SPE with 5 mL methanol then 5 mL chloroform.

Load 1 mL of filtrate sample.

Elute with 15 mL 5% methanol in chloroform.]

Blow down eluate to 1 mL.

Transfer 200 µL to 500 µL insert and dry under nitrogen.

Reconstitute with 100µL pyridine and 100 µL BSTFA:TMCS 99:1 and derivatize at 70C for 20 min.

### Wash:

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### Dry:

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### Elute:

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### Final Prep and Analysis:

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Initial Sample Weight / Unit: 0.70 grams

Inject: 0.5 µL on HPLC Flame Ionization (FID) @ (350°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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