

GC Application

ID No.: 16752

Sterols from Lard 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.

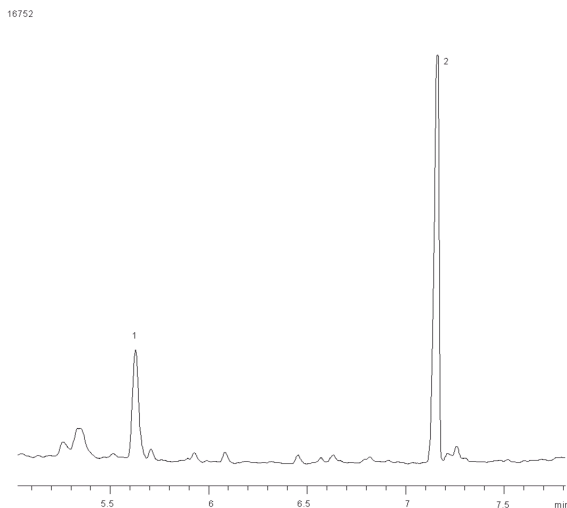
Recommended Accessories:

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 Cholesterol
- 2 Betulin (internal standard)



Sample Preparation Details

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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:

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:

Dry:

Elute:

Final Prep and Analysis:

Initial Sample Weight / Unit: 0.72 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 Cholesterol	0				
2 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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