APPLICATION NOTE
E184VPG-001

Separation of Cholesterol and its oxidation products

Abstract

Cholesterol is an important membrane lipid providing structural strength for the cell as well as regulating membrane fluidity. As one of the main constituents of lipid rafts, it is known to influence processes like signal transduction and membrane trafficking [1]. By partial depletion of cholesterol from membranes and subsequently substituting it with oxycholesterols, these functions can be studied more closely.
With this method, we are able to link cholesterol and oxycholesterol content in cell membranes to certain observations that are made in cell culture when for example varying oxycholesterol content in culture medium or challenging cells with stress conditions.

Keywords

- Natural products
- Sterols
- Oxysterols
- Cholesterol
- Cholesterol derivatives

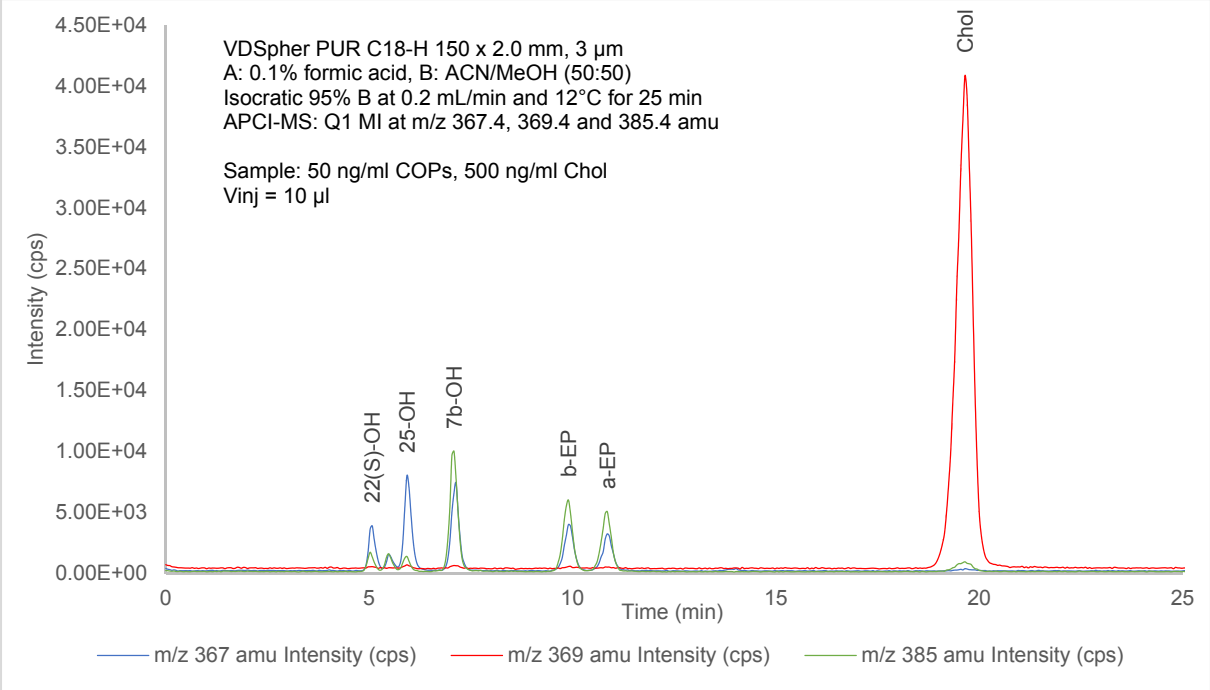
Compound information

Classification	Compound name
Sterol	Cholesterol
Oxysterols	22(S)-hydroxycholesterol 25-hydroxycholesterol 7-*beta*-hydroxycholesterol *beta*-epoxy-cholesterol *alpha*-epoxy-cholesterol

Chromatographic conditions

Column Particle Size, Length × inner diameter Order number	VDSpher® PUR 100 C18-H 3 µm, 150 × 2.0 mm N1520E184VPG
Separation mode descriptions	analytical, reverse phase
Mobile Phase	A: 0.1 % Formic acid, B: Acetonitrile/Methanol (50:50 v/v)
Elution conditions	Isocratic 0-25 min: 95% B
Flow rate	0.2 ml/min
Injection	10 µl
Column temperature	12°C
HPLC system	Shimadzu Prominence HPLC unit
Detector	ABSciex API 2000 mass spectrometer with APCI ion source; selected ion monitoring at m/z = 367, 369 and 385 amu
Sample and sample preparation	Sample: Lipid extract from cell membranes Preparation: cell membranes are solubilized by detergent (e.g. Triton X) and extracted with chloroform/methanol [2]. c = 500 ng/ml cholesterol, 50 ng/ml cholesterol oxidation products

Chromatograms



Origin

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