



BDG SYNTHESIS

Certificate of Analysis

BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

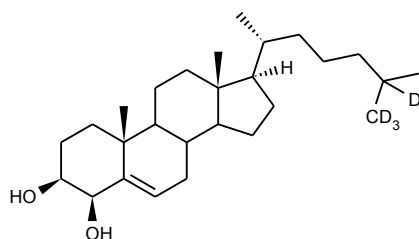
Barry Dent

Barry R. Dent, PhD, Director
24 July 2012

Name: 4 β -Hydroxycholesterol-d₄

CAS Number: 17320-10-4 (unlabelled)

Structure:



Molecular Weight: C₂₇H₄₂D₄O₂ = 406.68

Lot Number: BDG 13513.1

Appearance: Off-white, crystalline solid

Purity By HPLC: 97.2 %

Isotopic Purity: Under 0.5 % d₀

Re-test Date: 24 July 2017

Storage and Handling: Temperature: refrigerate for prolonged storage; may be handled and shipped at ambient temperature.

Humidity: not believed to be hygroscopic; may be handled in normal laboratory atmosphere.

Light: protect from strong sunlight.

Caution: only experienced laboratory personnel should handle the material.

Identity and Purity

Proton NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.

Isotopic Labelling: signals at the sites of deuteration are greatly diminished, compared with the spectrum of unlabelled material, indicating clean deuteration.

Residual Solvents: no residual solvents are observed.

Impurities: no significant impurities are evident in the spectrum.

Carbon-13 NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.

Isotopic Labelling: signals at the sites of deuteration have collapsed to small multiplets compared with the spectrum of unlabelled material, indicating clean deuteration.

High-resolution Mass Spectrum (ESI+)

Found m/z 429.3644. $C_{27}H_{42}D_4O_2Na$ $[M+Na]^+$ requires m/z 429.3647. The deviation of 0.7 ppm is within normally accepted limits for the establishment of identity by HRMS. A signal for d_0 material was observed at approximately the background level of 0.5 %, but analysis of both the NMR data and the molecular ion envelope indicates that this signal is most likely instrument noise.

HPLC

A somewhat broadened, symmetrical peak is observed (97.2 %). Note: in the absence of reference materials for preparing calibration curves, it is assumed that all peaks have the same detector response. Where possible, the conditions of analysis follow a pharmacopeial or literature method, or have been adapted from same.

Elemental Analysis

	Found:	C 79.66, H 10.56, D 2.01 %
$C_{27}H_{42}D_4O_2$	Requires:	C 79.74, H 10.41, D 1.98 %

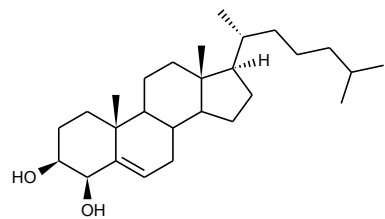
The elemental analyses fall within generally accepted limits for establishing the molecular formula given. The results may also be taken to imply the absence of significant quantities of water or inorganic salts (which have not been elsewhere tested for because of sample size limitations).

The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials often contain higher levels of residual solvents and/or water, and we urge you to use the corrected purity where needed rather than the raw HPLC purity. This compound is intended for use as an analytical reference material and it is not for human administration. Structures are shown with relative stereochemistry unless otherwise specified.

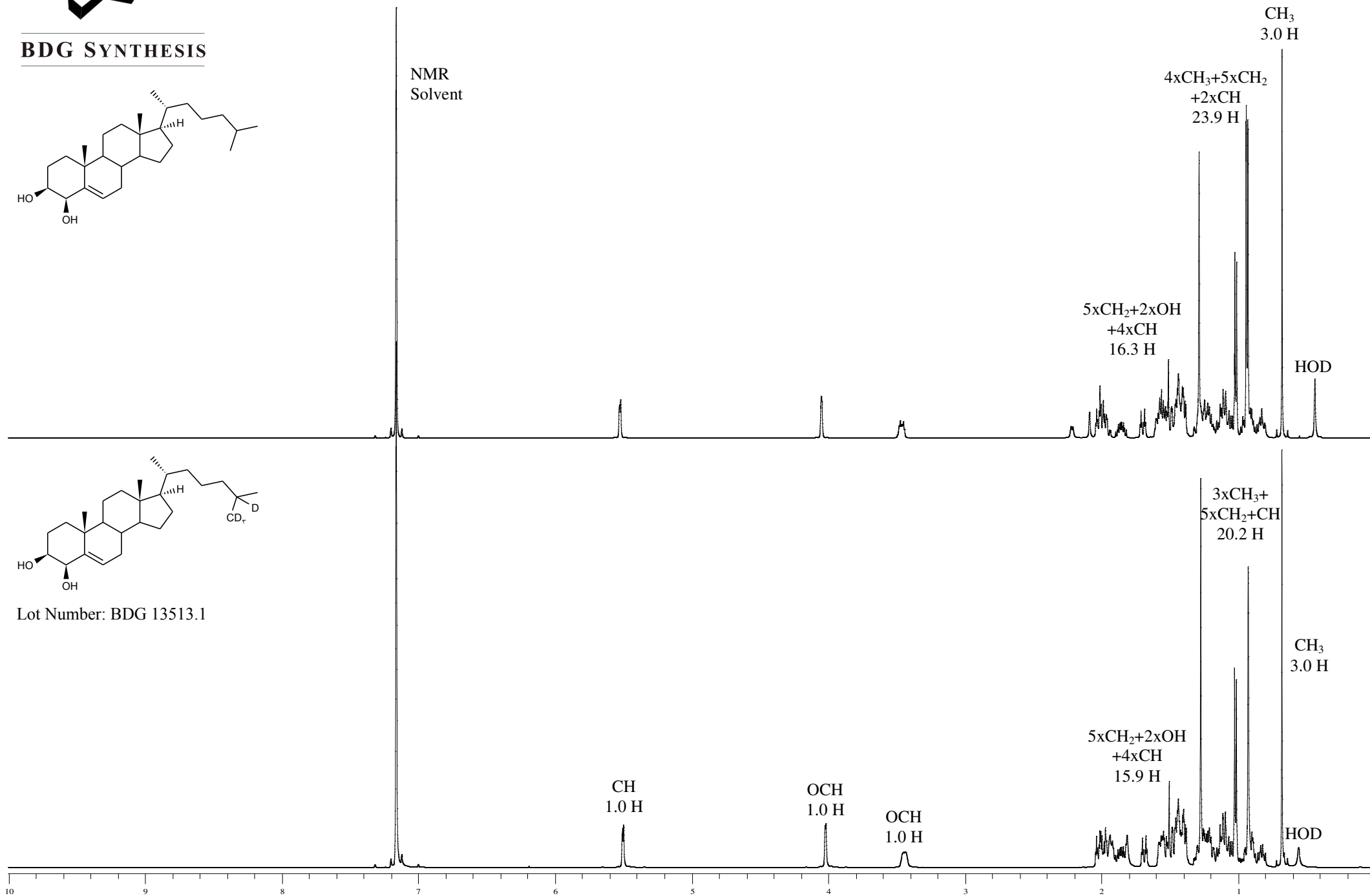
The re-test date is assigned from experience gained with the material in the laboratory and/or on storage. It is not possible to perform formal storage studies because of the small amount of material available.



BDG SYNTHESIS

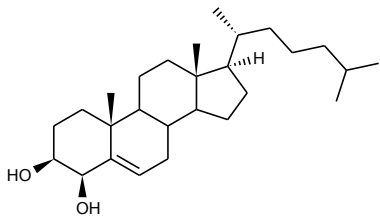


Proton NMR Spectrum of 4β-Hydroxycholesterol (top) and 4β-Hydroxycholesterol-d₄ (bottom) in C₆D₆

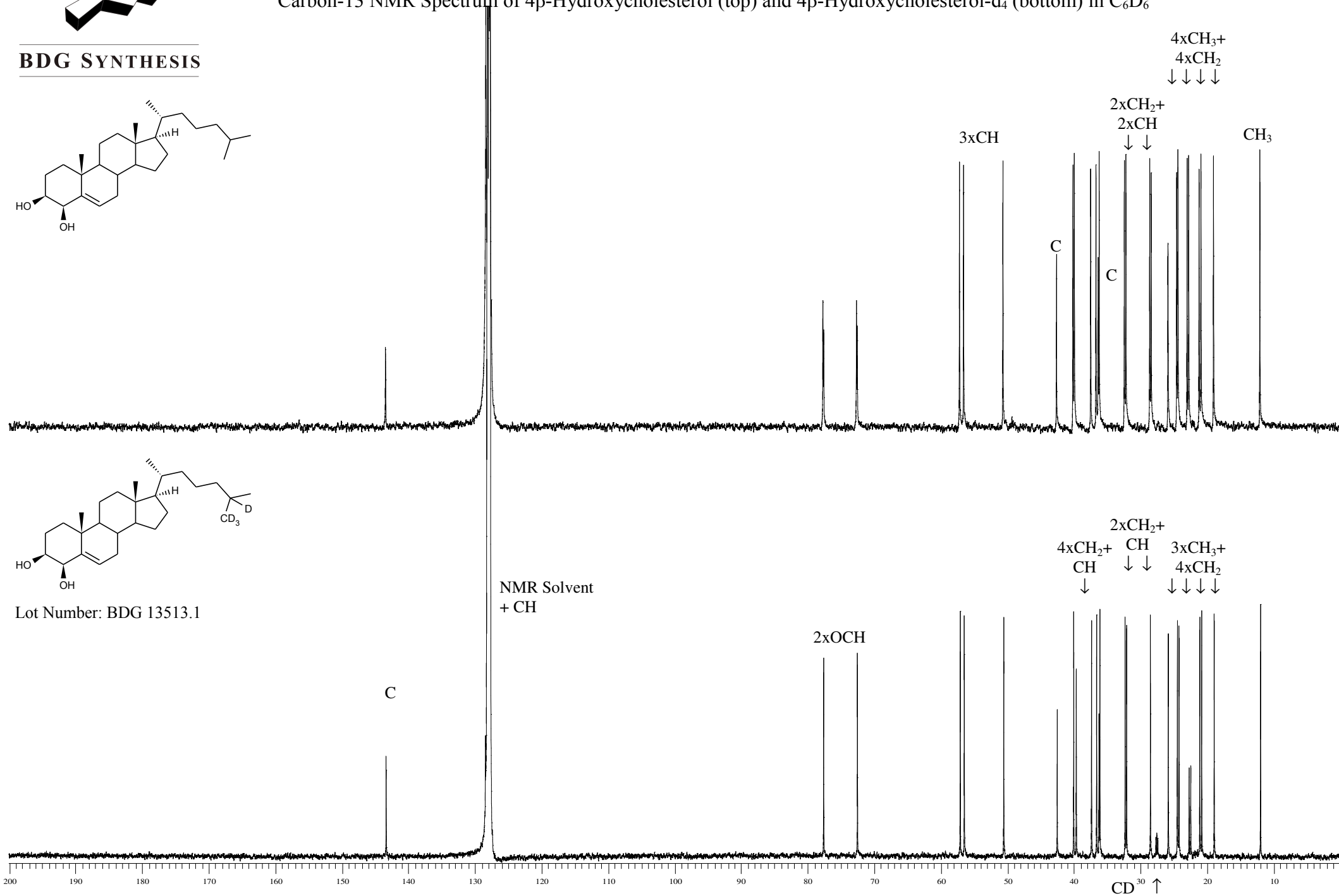




BDG SYNTHESIS



Carbon-13 NMR Spectrum of 4 β -Hydroxycholesterol (top) and 4 β -Hydroxycholesterol-d₄ (bottom) in C₆D₆

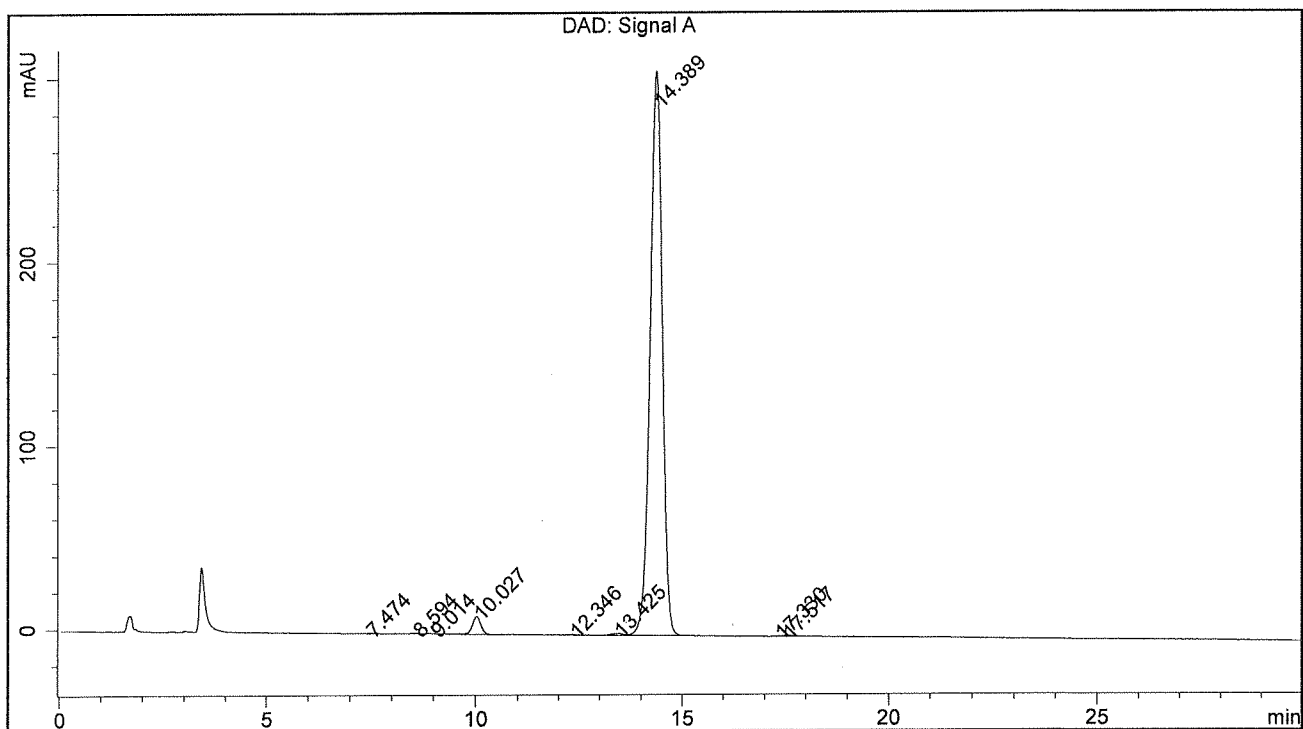


Lot Number: BDG 13513.1

BDG - Analysis of 4-beta-Hydroxycholesterol-d4

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
 Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
 Mobile Phase : 97 : 1.5 : 1.5 Methanol : Acetonitrile : Water
 Flow Rate : 1.0 mL/min Column Temperature : 20C
 Sample Solvent : Mobile Phase
 Injection Volume : 25 uL Detection : UV at 210 nm

Sample Name	BDG 13513.1	Instrument	AnalyticalLC01
Acquisition	24/07/2012, 14:30:33	Method (rev.)	LC10520f (2)
Sequence	BDG_24Jul2012c	Vial Position	41
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	7.47 min	0.3286	3.6051	0.1440 min	0.055 %
2	8.59 min	0.2661	2.1091	0.1065 min	0.032 %
3	9.01 min	0.2141	1.1698	0.0798 min	0.018 %
4	10.03 min	9.6494	136.0042	0.2144 min	2.090 %
5	12.35 min	0.4286	5.8144	0.1743 min	0.089 %
6	13.42 min	0.9911	22.0591	0.2717 min	0.339 %
7	14.39 min	307.6465	6321.4002	0.3177 min	97.163 %
8	17.33 min	0.2612	1.9414	0.0987 min	0.030 %
9	17.52 min	0.4891	11.8805	0.2954 min	0.183 %