BDG SYNTHESIS

Certificate of Analysis

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

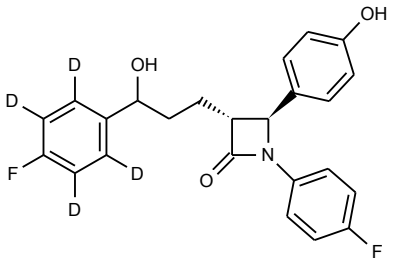
Barry Dent

Barry R. Dent, PhD, Director
26 July 2012

Name: **Ezetimibe-d_4**

CAS Number: 163222-33-1 ((3R)-unlabelled)

Structure:



Molecular Weight: $C_{24}H_{17}D_4F_2NO_3$ = 413.45

Lot Number: BDG 4868

Appearance: White, crystalline solid

Corrected Purity: 99.4 % (HPLC) - 0.7 % (ethyl acetate) - 0.9 % (water) = 97.8 %

Isotopic Purity: Under 0.5 % d_0

Re-test Date: 26 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. Slight differences are observed compared with the spectrum of the enantiomerically pure unlabelled analogue, due to the presence of diastereoisomers.
Isotopic Labelling: signals at the sites of deuteration are greatly diminished, compared with the spectrum of unlabelled material, indicating clean deuteration.
Residual Solvents: a small amount of ethyl acetate (0.7 % *w/w*) and traces (under 0.1 % *w/w*) of hexanes and 2-propanol 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* 414.1816. $C_{24}H_{18}D_4F_2NO_3$ $[M+H]^+$ requires *m/z* 414.1819. The deviation of 0.7 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for d_0 material was seen (detection limit about 0.5 %).

HPLC

A sharp, symmetrical peak is observed (99.4 %). 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 69.21, H 4.22, D 1.98, N 3.31 %
$C_{24}H_{17}D_4F_2NO_3 \cdot 0.2H_2O$	Requires:	C 69.12, H 4.21, D 1.93, N 3.36 %, H_2O 0.86 %
$C_{24}H_{17}D_4F_2NO_3$	Requires:	C 69.72, H 4.14, D 1.95, N 3.39 %

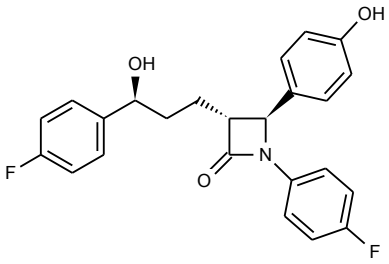
The elemental analyses fall slightly outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the “best-fit” hydrated molecular formula is given. In the absence of a Karl-Fischer water analysis, we recommend that the “best-fit” water content be used when determining corrected purity.

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.

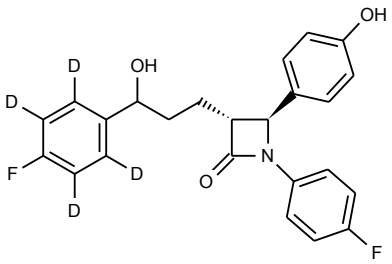
The re-test date is assigned from experience gained with the material in the laboratory and/or on storage. It is not possible to perform formal storage studies because of the small amount of material available.

BDG SYNTHESIS

Proton NMR Spectrum of Ezetimibe (top) and Ezetimibe-d_4 (bottom) in Methanol-d_4



12xCH
12.0 H



Lot Number: BDG 4868

8xCH
8.0 H

HOD

NMR
Solvent

OCH+NCH
2.1 H

ethyl
acetate
0.7% w/w

CH
1.0 H

2xCH$_2$+
ethyl acetate
4.2 H

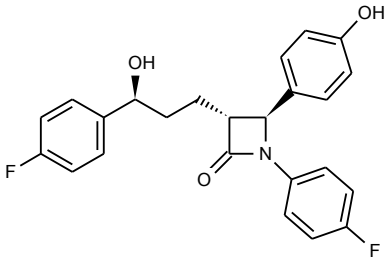
ethyl
acetate

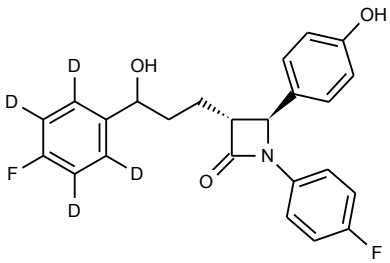
hexanes
↓

9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Ezetimibe (top) and Ezetimibe-d_4 (bottom) in DMSO-d_6





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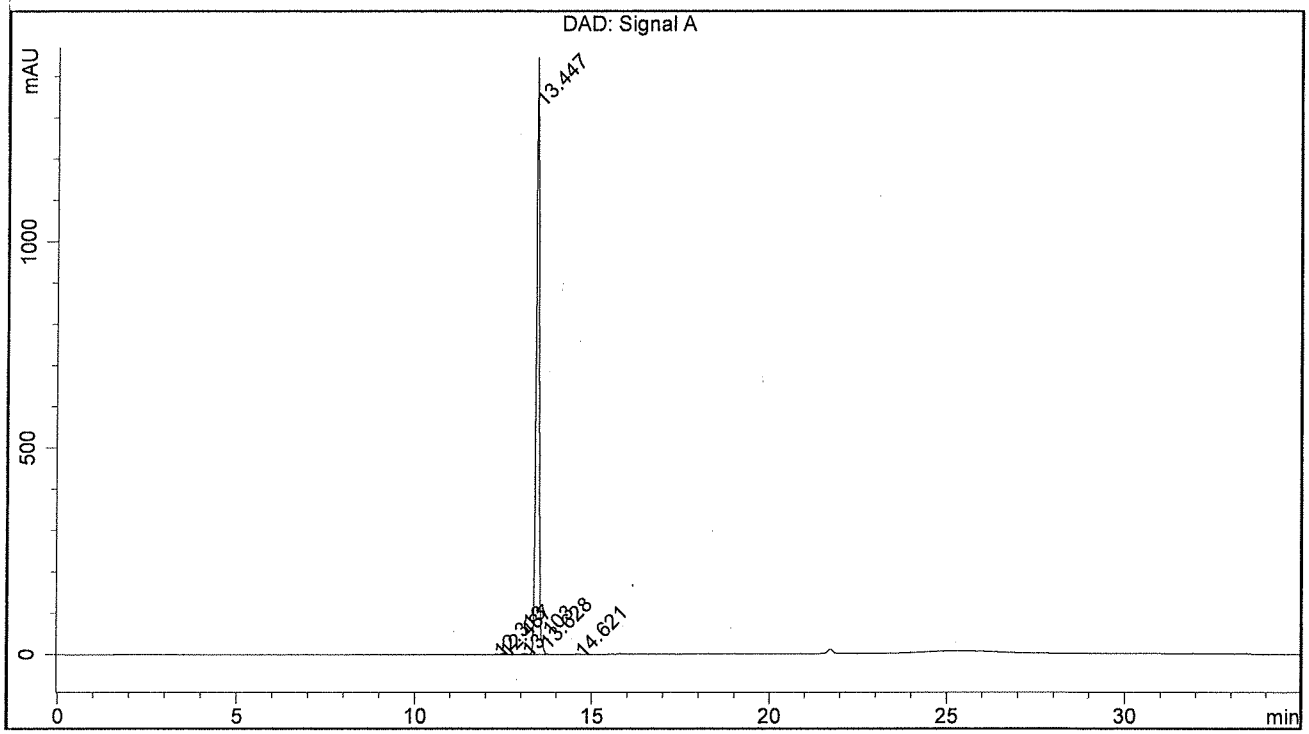
BDG - Analysis of Ezetimibe-d4

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase A : 70:30 20 mM Ammonium Acetate (pH=7.0) : Acetonitrile
Mobile Phase B : 20:80 20 mM Ammonium Acetate (pH=7.0) : Acetonitrile
Gradient (A:B) : T0=100:0, T15=0:100, T30=0:100, T32=100:0, T35=100:0
Flow Rate : 1.0 mL/min . . . . . Sample Solvent : Initial Mobile Phase
Column Temperature : 20C . . . . . Injection Volume : 10 uL . . . . . Detection : UV at 245 nm

Sample Name	BDG 4868	**Instrument**	AnalyticalLC01
Acquisition	26/07/2012, 18:53:38	**Method (rev.)**	LC10528a (5)
Sequence	BDG_26Jul2012d - Reprocessed	**Vial Position**	74
Operator	solvation010\cerityadmin	**Injection**	2 of 2



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	12.31 min	1.3018	7.5569	0.0917 min	0.083 %
2	12.49 min	2.8654	21.7179	0.1124 min	0.238 %
3	13.10 min	2.5217	15.0339	0.0956 min	0.164 %
4	13.45 min	1445.8174	9086.6137	0.0993 min	99.383 %
5	13.63 min	1.6340	3.6590	0.0381 min	0.040 %
6	14.62 min	1.2404	8.4870	0.1058 min	0.093 %