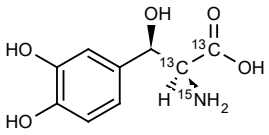
BDG SYNTHESIS

Certificate of Analysis

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

Neil Beare, PhD, Director
25 July 2018

Name:	**Droxidopa-$^{13}C_2$,^{15}N**	
CAS Number:	23651-95-8 (unlabelled)	
Structure:		
Molecular Weight:	$C_7{}^{13}C_2H_{11}{}^{15}NO_5$ = 216.17	
Lot Number:	BDG 17353.2	
Appearance:	White powder	
Corrected Purity:	99.8 % (HPLC) - 1.6 % (ethanol) - 0.2 % (methanol) - 4.0 % (water) – 1.0 % (triethylamine hydrochloride) = 93.0 %	
Isotopic Purity:	Under 0.5% M-3	
Re-test Date:	25 July 2023	
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. The material decomposes in solution over time.

• Custom synthesis of analytical reference standards, metabolites, stable isotope labelled compounds • Contract research • BDG Synthesis is a division of B Dent Global Limited

Mailing:
BDG Synthesis
PO Box 38 627, Wellington Mail Centre,
Wellington, New Zealand.

Shipping:
BDG Synthesis
Gracefield Research Centre, Building F,
Gracefield Road, Lower Hutt, New Zealand.

Phone: + 64 4 569 0520
Fax: + 64 4 569 0521

info@bdg.co.nz
www.bdg.co.nz

Identity and Purity

Proton NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.
Isotopic Labelling: the spectrum is of little value in determining isotopic purity although splitting is observed due to ^{1}H-^{13}C coupling.
Residual Solvents: small amounts of methanol (0.2 % *w/w*) and ethanol (1.6 % *w/w*) are observed.
Impurities: triethylamine hydrochloride (1.0 % w/w) is 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: the spectrum is of little value in determining isotopic purity, although the signals at the ^{13}C-labelled sites are massively enhanced as expected.
Impurities: minor ^{13}C-labelled impurities are evident in the spectrum.

High-resolution Mass Spectrum (TOF MS ES+)

Found *m/z* 239.0563. $C_7{}^{13}C_2H_{11}{}^{15}NNaO_5$ $[M+Na]^+$ requires *m/z* 239.0572. The deviation of 3.8 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for M-3 material was seen (detection limit about 0.5 %).

HPLC

A sharp, symmetrical peak is observed (99.8 %). 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 48.85, H 5.61, N 6.32 %
$C_7{}^{13}C_2H_{11}{}^{15}NO_5 \cdot 0.5H_2O$	Requires:	C 48.89, H 5.37, N 6.66 %, H_2O 4.00 %
$C_7{}^{13}C_2H_{11}{}^{15}NO_5$	Requires:	C 50.92, H 5.13, N 6.94 %

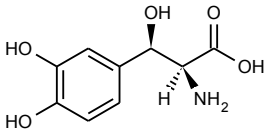
The elemental analyses fall somewhat outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the “best-fit” hydrated molecular formula is given. In the absence of a Karl-Fischer water analysis, we recommend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

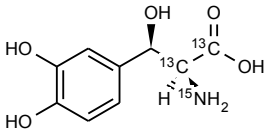
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials 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 Droxidopa (top) and Droxidopa-$^{13}C_2$,^{15}N (bottom) in DMSO-d_6

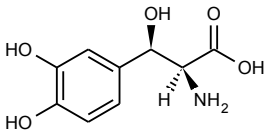


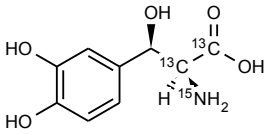


Lot Number: BDG 17353.2

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Droxidopa (top) and Droxidopa-$^{13}C_2$,^{15}N (bottom) in DMSO-d_6

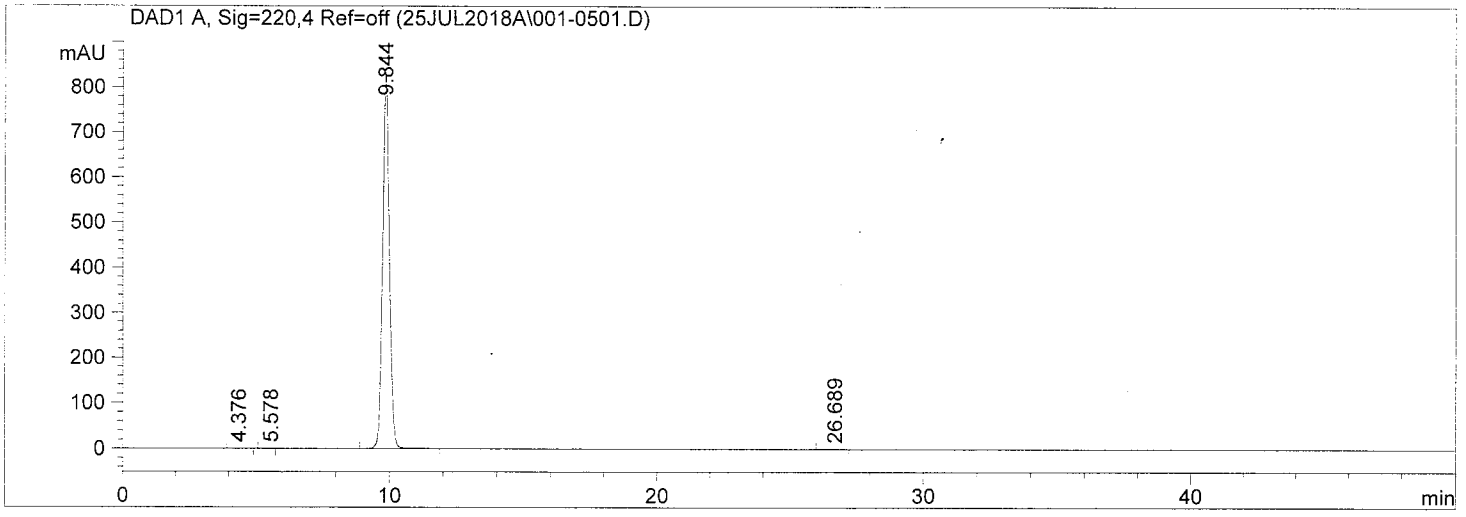




Lot Number: BDG 17353.2

Sample Name: BDG 17353.2

Injection Date : 7/25/2018 1:40:48 PM Inj : 1
Inj Volume : 10 µl
Acq. Method : C:\CHEM32\1\METHODS\2018\LC20019A.M
Last changed : 7/25/2018 12:08:07 PM by Bruce Hamilton
(modified after loading)
Analysis Method : C:\CHEM32\1\METHODS\2018\LC20019A.M
Last changed : 7/25/2018 4:39:11 PM by Bruce Hamilton
Method Info : BDG - Analysis of Droxidopa-13C2,15N
Column : Phenomenex Luna C18(2) 5 um 250 x 4.6 mm : Guard C18RP 4 x 3 mm
Mobile Phase : 93.5:6.5 5mM Sodium Heptanesulphonate in 10mM Potassium diHydrogen Phosphate pH=2.00 (H3PO4) : Acetonitrile
Flow : 1 ml/min., Column Temperature : 20 C, Injection : 10 ul,
Detection : UV at 220 nm, Sample Solvent : Mobile Phase



Area Percent Report

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=220,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.376	BB	0.3272	9.22186	3.54874e-1	0.0639
2	5.578	BB	0.1801	5.20840	3.89232e-1	0.0361
3	9.844	BV	0.2498	1.43999e4	832.46478	99.7802
4	26.689	BV	0.4328	17.28875	4.91040e-1	0.1198
Totals :				1.44316e4	833.69993	

*** End of Report ***